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CONTRACT NO: DAMD17-91-C-1001

TITLE: MOLECULAR STUDIES OF HTLV-I INFECTION IN NEWLY RECOGNIZED HIGH RISK POPULATION

PRINCIPAL INVESTIGATOR: Yehuda Danon, M.D.

CONTRACTING ORGANIZATION: Tel-Aviv University
Beilinson Medical Center
Petah-Tikva, 49100, Israel

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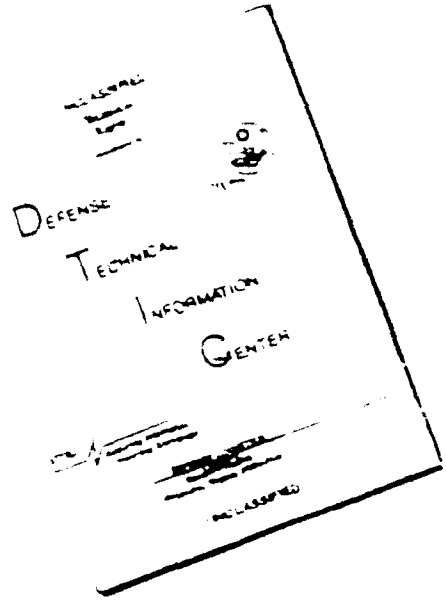

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13. ABSTRACT *(Maximum 200 words)*

The report summarizes the two years of contract of the research aimed to define newly recognized high risk population for HTLV-I infection. To define the extent of HTLV-I infection among groups of Jewish immigrants to Israel with an increased frequency of Adult T-cell Leukemia (ATL). Various serological and molecular screening of methods were used, including ELISA and PCR use for molecular survey by amplification of HTLV-I proviral DNA from prripheral blood mononuclear cells DNA. Overall rate of infection is 12% for Jews arriving from Khurusan-North-Eastern Iran. No positive HTLV-I carries were found from other parts of Iran, Ethiopia, and other Mid-Eastern countries, We could not identify HTLV-I seropositive patients in Insulin Dependent Diabetes Mellitus (IDDM) patients in Children with non-Burkitt lymphomas, Psoriasis and Parapsoriasis patients, DNA Sequence data for HTLV-I isolates are provided showing similar sequence to African isolates.

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97

HTLV-I INDUCED T-CELL LYMPHOMA IN ISRAELI PATIENTS OF IRANIAN ORICIN

J. Rosenblatt, D. Meites, Y. Sidi, Y.L. Danon

Rogoff-Wellcome Medical Research Institute, Dept. of Medicine and Div. of Pediatric Immunology, Edith Wolfson Hospital and Beilinson Medical Center Tel-Aviv University Sackler School of Medicine, Petah-Tikva 49100 ISRAEL.

T cell lymphoma-leukemia (ATL) is one of the several clinical entities linked to human T cell lymphotropic virus (HTLV-I). Few geographic endemic concentrations of HTLV-I infection were already described: The Ryukyu Islands in Southern Japan, Central Africa and the Caribbean Islands. This is the first description of endemic HTLV focus in the Middle East. The prevalence and clinical presentations of ATL in Israel were studied. We have diagnosed four Israeli Jewish ATL patients and one HAM (HTLV-I - Associated Myelopathy) in a nationwide survey performed in 1986-1990. In three of the patients evidence for HTLV-I infection was obtained. All those patients immigrated to Israel from the same region in Central Iran. The nationwide survey and the clinical course of this new group of patients will be presented.

Japan

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MOLECULAR CHARACTERIZATION OF IRANIAN HTLV-I ISOLATES

Y. Kilim[1], J.D. Rosenblatt[2], D. Meytes[3], D. Stephens[2], H. Lee[4], Y. Danon[1]
Children's Medical Center of Israel, Petach, Tiqva, Israel[1], UCLA School of Medicine, Los Angeles, CA[2], Edith Wolfson Hospital, Holon, Israel[3], Abbott Laboratories, N. Chicago, IL[4], USA

We recently reported a high rate of HTLV-I seropositivity among immigrants to Israel from Mashad in Northeastern Iran (Lancet 336:1533-1535, 1990). We have now characterized the Iranian HTLV-I isolate using a combination of Southern blotting, polymerase chain reaction (PCR) and sequencing. DNA from 10 HTLV-I seropositive Mashadi carriers was isolated from peripheral blood mononuclear cells and amplified by PCR using primers to the tax/rex region. All 10 samples were found to be HTLV-I and not HTLV-II using discriminatory PCR. An immortalized T-cell line was derived from a Mashadi HTLV-I carrier. The cell line contained integrated HTLV-I provirus. Southern blotting and restriction mapping of the Iranian isolate demonstrated marked similarity to published Japanese isolates using 10 different restriction enzymes. Amplification and preliminary sequencing of a 900 bp segment of env from two Mashadi isolates disclosed approximately 95% nucleic acid sequence homology with the published sequence of Japanese isolates, and >98% homology between two Mashadi isolates. These data indicate substantial conservation of sequence between Iranian and Japanese HTLV-I isolates. Further characterization of Iranian HTLV-I isolates is underway. (Supported by the Rashi Foundation, Doron Foundation, ICRF, and a grant from the USAMRDC).

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EPIDEMIOLOGIC AND MOLECULAR CHARACTERIZATION OF HTLV-I INFECTION IN ISRAEL

Yehuda L. Danon, Yael Kilim and J. Rosenblatt, Kipper Institute of Child Immunology, Children's Medical Ctr. of Israel, Sackler School of Medicine, Tel-Aviv Univ. Kaplan Str. #14-16, Petah-Tikva 49100 Israel.

Human T-cell leukemia viruses type I (HTLV-I) and type II (HTLV-II) are the two human retroviruses directly implicated in pathogenesis of human leukemia. HTLV-I has been linked to adult T-cell leukemia/ lymphoma (ATLL), and HTLV-II to rare cases of chronic T-cell leukemia. Epidemiologic and molecular studies of both viruses have identified several themes underlying the leukemogenic process. Leukemia is a rare consequence of infection, and both viral infection and secondary transforming events appear to be involved. Due to the absence of a virally encoded oncogene, or preferred viral integration sites, novel mechanisms of leukemogenesis must be invoked. We recently reported a high rate of HTLV-I seropositivity among immigrants to Israel from Mashad in Northeastern Iran (Lancet 336:1533-1535,1990). We have now characterized the Iranian HTLV-I isolate using a combination of Southern blotting, polymerase chain reaction (PCR) and sequencing. DNA from 10 HTLV-I seropositive Mashadi carriers was isolated from peripheral blood mononuclear cells and amplified by PCR using primers to the tax-rex region. All 10 samples were found to be HTLV-I and not HTLV-II using discriminatory PCR. An immortalized T-cell line was derived from a Mashadi HTLV-I carrier. The cell line contained integrated HTLV-I provirus. Southern blotting and restriction mapping of the Iranian isolate demonstrated marked similarity to published Japanese isolates using 10 differrent restriction enzymes. Amplification and preliminary sequencing of a 900 bp segment of env from two Mashadi isolates disclosed approximately 95% nucleic acid sequence homology with the published sequence of Japanese isolates, and >98% homology between two Mashadi isolates. These data indicate substantial conservation of sequence between Iranian and Japanese HTLV-I isolates. Further characterization of Iranian HTLV-I isolates is underway. A potential role for the HTLV-I/II transregulatory proteins Tax and Rex in leukemogenesis is discussed. Insights derived from the study of HTLV-I/II can be applied to the search for other oncogenic viruses.

(Supported by the Rashi Foundation, Doron Foundation, ICRF, and a grant from the USAMRDC).

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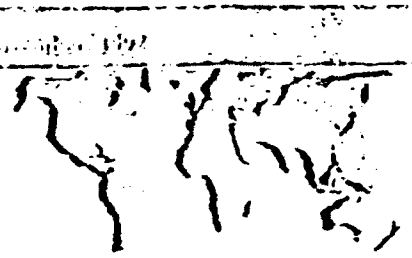

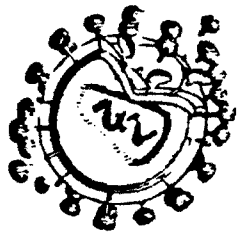

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EPIDEMIOLOGIC AND MOLECULAR CHARACTERIZATION OF HTLV-I INFECTION IN ISRAEL

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Viruses and Virus-Like Agents in Disease

Molecular characterization of HTLV-I infection in Israel

Yehuda L. Danon, Yael Kilim and Joseph Rosenblatt
Kipper Inst. of Child Immunology, Children's Med. Ctr. of Israel, Sackler School of Med., Tel-Aviv Univ. Israel

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MOLECULAR CHARACTERISTICS OF HTLV-I INFECTION IN NEWLY CHARACTERISED HIGH RISK GROUP OF CARRIERS IN THE MIDDLE EAST.

HTLV-I has been linked to adult T-cell leukemia/lymphoma (ATLL), and HTLV-II to rare cases of chronic T-cell leukemia. We recently reported a high rate of HTLV-I seropositivity among immigrants to Israel from Mashad in Northeastern Iran. We have now characterized the Iranian HTLV-I isolate using a combination of Southern blotting, polymerase chain reaction (PCR) and sequencing. DNA from 10 HTLV-I seropositive Mashadi carriers was isolated from peripheral blood mononuclear cells and amplified by PCR using primers to the tax-rex region. All 10 samples were found to be HTLV-I and not HTLV-II using discriminatory PCR. The cell line contained integrated HTLV-I provirus. Southern blotting and restriction mapping of the Iranian isolate demonstrated marked similarity to published Japanese isolates using 10 different restriction enzymes. Amplification and preliminary sequencing of a 900 bp segment of env from two Mashadi isolates disclosed approximately 95% nucleic acid sequence homology with the published sequence of Japanese isolates, and >98% homology between two Mashadi isolates. These data indicate substantial conservation of sequence between Iranian and Japanese HTLV-I isolates. Insights derived from the study of HTLV-I/II can be applied to the search for other retroviruses and oncogenic viruses.

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Blood

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(Supported by the USAMRDC and Doron Foundation)

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MOLECULAR CHARACTERIZATION OF HTLV-I INFECTION IN ISRAEL

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HTLV-I VIRUS IN INSULIN-DEPENDENT DIABETES MELLITUS

Y. Kilim, M.Sc.[1], M. Karp, M.D.[2] and Y.L. Danon, M.D.[1]
[1]Kipper Institute of Immunology,
[2]Institute of Pediatric and Adolescent Endocrinology,
The Children's Medical Center of Israel, Petah-Tikva, Israel

Human T-cell Leukemia Virus-I has been linked to adult T cell leukemia/lymphoma (ATLL) and HTLV-II to some cases of chronic T cell leukemia. We have recently reported a high rate of HTLV-I sero-positive among immigrants to Israel from northeastern Iran, and especially the town of Mashad.

To determine the frequency of antibodies to HTLV-I virus in Insulin-Dependent Diabetes Mellitus (IDDM) patients, sera from 56 newly onset IDDM patients were tested by an enzyme immunoassay. According to our method the reactivity of antibodies detected by enzyme immunoassay against HTLV-I encoded antigens was determined by an assay which employs recombinant HTLV-I antigens. No antibodies to HTLV-I were detected in all 56 patients studied. Proliferative response to various species of insulin was performed in 26 of those patients, 23 out of 26 showed a positive response. Sera from 56 newly onset IDDM patients were screened for ICA. ICA were detected in 32 (57.1%) of the 56 patients.

It seems that HTLV-I is playing no role in IDDM.

Supported in part by USAMRDC Grant # DAMD17-91-C-1001

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EPIDEMIOLOGIC AND MOLECULAR CHARACTERIZATION OF NEW HTLV-I INFECTION FOCUS IN THE MIDDLE EAST

Yehuda L. Danon, Yael Kilim and Joseph Rosenblatt, Kipper Institute of Child Immunology, Children's Medical Center of Israel, Sackler School of Medicine, Tel-Aviv University Israel

HTLV-I has been linked to adult T-cell leukemia/lymphoma (ATLL), and HTLV-II to rare cases of chronic T-cell leukemia. We recently reported a high rate of HTLV-I sero-positivity among immigrants to Israel from Mashad in Northeastern Iran after a national serologic survey of blood donors. We have now characterized the Iranian HTLV-I isolate using a combination of Southern blotting, polymerase chain reaction (PCR) and sequencing. DNA from 17 HTLV-I seropositive Mashadi carriers was isolated from peripheral blood mononuclear cells and amplified by PCR using primers to the tax-rex region. All 17 samples were found to be HTLV-I and not HTLV-II using discriminatory PCR. The cell line contained integrated HTLV-I provirus. Southern blotting and restriction mapping of the Iranian isolate demonstrated marked similarity to published Japanese isolates using 10 different restriction enzymes. Amplification and preliminary sequencing of a 900 bp segment of env from two Mashadi isolates disclosed approximately 95% nucleic acid sequence homology with the published sequence of Japanese isolates, and >98% homology between two Mashadi isolates. These data indicate substantial conservation of sequence between Iranian, African (Zair) and Japanese HTLV-I isolates. Insights derived from the study of HTLV- I/II can be applied to the search for other retroviruses and oncogenic viruses.
(Supported by the USAMRDC and Doron Foundation)

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KEY WORDS
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A Decade with HTLV-I/HTLV-II: Lessons in Viral Leukemogenesis

Joseph D. Rosenblatt[1], Yehuda. Danon[2], and Alexander C. Black[1]

[1]Division of Hematology-Oncology, Department of Medicine and Jonsson Comprehensive Cancer Center, UCLA School of Medicine, Los Angeles, CA, USA and the [2]Kipper Institute of Child Immunology, Children's Medical Centre of Israel, Beilinson Medical Center, Petach Tiqva, Israel

INTRODUCTION

The past decade has seen myriad advances in detection and characterization of human retroviruses. It began with initial description of human T-cell leukemia virus type I (HTLV-I) by Poiesz and Gallo in the US and Yoshida in Japan, which pointed to the involvement of the human retrovirus, HTLV-I, in an unusual form of T-cell malignancy, adult T-cell leukemia/lymphoma (ATLL) (1,2). The identification of HTLV-I intensified the search for related viruses, and soon thereafter, human T-cell leukemia virus type II (HTLV-II) was described by Kalynaraman and Gallo in a cell line derived from a patient with a chronic T-cell leukemia with features of hairy-cell leukemia (3). The rapid identification of HTLV-II on the heels of HTLV-I led to speculation that a host of human oncogenic retroviruses would soon be identified. The subsequent discovery of human immunodefiency virus types 1 (HIV-1) and 2 (HIV-2) and their implication in acquired immunodeficiency syndrome (AIDS) accelerated the pace and intensity of the search for oncogenic viruses. It was soon recognized that leukemic cells in malignancies associated with HTLV-I and -II contained clonally integrated provirus; in effect, a signature for direct viral involvement in the oncogenic process. In contrast, neoplasms frequently seen in the setting of HIV-1 infection (e.g. Kaposi's sarcoma and/or high-grade B-cell lymphomas) did not appear arise as a direct consequence of viral transformation of HIV-1-infected cells. At the end of the decade, only HTLV-I and -II remain clearly implicated as directly leukemogenic human retroviruses. Therefore, we believe that insights gleaned from investigation of these viruses can and should be applied to the search for other oncogenic retroviruses.

EPIDEMIOLOGICAL LESSONS

The epidemiological link between HTLV-I and ATLL resulted from two complementary events. The first, an appreciation by Uchiyama and Takatsuki that ATLL represented a unique clinical entity (4) allowed geographic localization of the disease to southern islands of Japan: Kyushu, Shikoku, and the Ryuku chain of islands. Development of serological assays for HTLV-I led to correlation of HTLV-I infection to the presence of malignancy, as well as a determination of modes of transmission (for review see 5). Epidemiological studies have suggested that exposure shortly after birth is a major risk factor for subsequent development of ATLL (5,6). In addition, these studies have demonstrated that twenty or more latent years may elapse between acquisition of infection and development of malignancy (5,6). Furthermore, only a minority (< 5%) of HTLV-I carriers actually develop ATLL (7), and ATLL as a consequence of transfusion-acquired HTLV-I is virtually unknown.

Hence, several general observations emerged from scrutiny of HTLV-I epidemiology: (a) leukemia may be an infrequent consequence of exposure to a fairly wide-spread virus; (b) leukemogenesis may depend on the timing and/or length of exposure, so that individuals infected in childhood may be at higher risk than those infected later in life; and (c) the long latency period suggests a multiple step process may be involved in leukemogenesis; while viral infection may be a prerequisite, it alone may be insufficient to produce the leukemic phenotype. These general epidemiologic features of ATLL suggest that a systematic re-evaluation of appropriate candidate malignancies and viruses may reveal other oncogenic viruses. Fairly prevalent or even ubiquitous viruses could conceivably manifest oncogenic potential in a sporadic fashion, and factors such as timing and length of exposure may be critical.

Careful cataloguing and description of clinical syndromes is essential to derive epidemiologic clues that may lead to virus identification. The recognition that non-Hodgkin's lymphomas could be divided into T- and B-cell subtypes and subsequent differentiation of ATLL from *mycosis fungoides* is a case in point. While ATLL was undoubtedly a frequent reason for in-patient hospitalizations in Japan prior to 1977, it was thought to be a variant of peripheral cutaneous T-cell lymphoma, and its characteristic features such as hypercalcemia and enhanced expression of interleukin 2 (IL-2) receptor alpha (IL-2Rα) chain (Tac antigen) on the cell surface were initially overlooked. Recognition of the subtler clinical aspects of the syndrome allowed for subsequent epidemiologic observations (4).

In contrast to HTLV-I, it is premature to reach conclusions regarding pathogenesis by HTLV-II. Although originally isolated from the Mo T-cell line, a transformed T-cell line derived from the spleen of a patient with hairy-cell leukemia, the nature of the malignancy *in vivo* in the patient was not adequately addressed (8). We know that HTLV-I and -II can transform T-cell lines *in vitro*, and that the Mo T-cell line may have simply represented an outgrowth of HTLV-transformed cells *in vitro*. A second patient with HTLV-II and hairy-cell leukemia was found by our laboratory to have a biclonal lymphoproliferative

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disorder in which a B-cell hairy-cell leukemia and a co-existant malignant CD8+ T-cell clone were observed (9,10). Oligoclonal integration of HTLV-II provirus into the CD8+ T-cells provided strong evidence for origin of malignancy in a virally infected cell. However, as additional cases of HTLV-II-induced malignancy have not been reported, there is considerable doubt as to whether we have as yet characterized the prototypic disease associated with HTLV-II.

An additional surprise that has emerged from epidemiological studies of HTLV has been the fact that screening procedures for HTLV-I identify a considerable number of crossreactive HTLV-II carriers. This raises the possibility that in the process of assaying for newly identified viruses, we may inadvertantly be assaying for a variety of crossreactive members of the same viral family. Specifically, intravenous drug abusers (IVDA) found to be seropositive for HTLV-I have been reported in several studies to have a higher incidence of HTLV-II infection and > 50% of seropositive random blood donors screened by HTLV-I ELISA were actually found by DNA amplification techniques to harbour HTLV-II (11–13). In the future, screening for newly identified viruses should be performed using both DNA amplification and serological techniques to avoid the initial confusion in delineating the epidemiology of HTLV-I and -II.

The HTLV-I model of malignancy as a rare consequence of infection with a prevalent virus suggests that careful molecular re-examination of the role of already recognized viruses in suspect malignancies is in order. In the case of HTLV-I, over a million infected individuals in Japan give rise to only 400–500 cases of ATLL per year. Hodgkin's disease (HD) may provide another case in point. The bimodal age distribution, prevalence, anecdotal descriptions of geographical clustering, and 'outbreaks' of HD suggest that an infectious agent may underlie pathogenesis (14–21). The increasingly frequent reports of Epstein-Barr virus (EBV) genome detection in some cases of HD suggest that EBV can be important in pathogenesis of a subset of HD patients (14–21). As another example, recognition that four of the last five cases of ATLL-like T-cell lymphoma in Israel occurred in Iranian immigrants from the northeastern city of Mashad, allowed identification of a new focus of HTLV-I infection (22,23). Recognition of geographic, familial and/or ethnic clustering of particular malignant disorders may yield important clues to viral etiology. It is important to note, however, that such time/space clustering may frequently relate to non-infectious risk factors rather than a virus. Some investigators have speculated on the likelihood of viral involvement in childhood acute lymphoblastic leukemia (ALL); however, the evidence is only mildly suggestive at best. Some studies suggest an association with geographic areas of high socioeconomic status, while others do not (24–29). Additional studies suggest a mild degree of clustering, particularly in children less than six years of age, although this remains controversial (29–31). Thus, little evidence points to an infectious cause or an underlying common leukemia virus in ALL. If a virus were involved, analogy to ATLL would suggest that it might initially cause an insignificant acute infection that establishes latency and eventually leads to leukemia through secondary events. Given the lack of overt clustering, seroepidemiological studies are unlikely to settle the issue, and frank demonstration of molecular involvement of an infectious agent will likely be necessary.

New Molecular Mechanisms of Pathogenesis

The explosive growth in the study of oncogenes over the past decade came about as a result of recognition that in animal malignancies brought about by retroviral infection, the transduction of a cellular proto-oncogene and its inappropriate expression under control of the viral promoter was frequently observed in retrovirally induced tumors. A second type of molecular lesion frequently observed was integration of a retrovirus adjacent to a cellular oncogene and loss of normal patterns of proto-oncogene expression. These observations led to speculation that similar mechanisms may be operative in human malignancy. However, HTLV-I afforded a unique surprise, in that the viral sequences did not contain any transduced cellular sequences, and that integration sites appeared to be random. While clonal integration was observed by Yoshida and colleagues, the sites of integration often occurred on different chromosomes, and no specific integration patterns could be observed (2,32). These observations led to a search for new mechanisms of oncogenesis.

The demonstration by Seiki and colleagues of the unique coding regions located in the 3' end of the genome provided a first clue as to potential mechanisms (33). The 3' ends of the genome of HTLV-I, -II and the leukemogenic bovine leukemia virus (BLV) all contain coding sequences for at least two overlapping genes (34–37). These genes, known as *tax* and *rex*, are now known to encode a transcriptional and post-transcriptional regulator of viral expression. The initial discovery of the *tax* gene was surprising, in that such *trans*-acting transcriptional regulators had only been previously identified in DNA viruses such as herpes simplex virus, varicella-zoster virus, and adenovirus. The HTLV-I *tax* gene encodes a 40-kilodalton (kDa) protein, and the HTLV-II *tax* gene encodes a 37-kDa protein (34–37). These nuclear phosphoproteins are highly homologous, and have similar effects on transcription. Tax expression is necessary for transcriptional activation of virus replication and efficient RNA production from the viral long terminal repeat (LTR); however, an additional property noted early was the ability of Tax to *trans*-activate other viral and cellular promoters, including those involved in regulation of T-cell growth. Examples of such promoters include those for IL-2, IL-2Rα, and the lymphokine, granulocyte-macrophage colony-stimulating factor (GM-CSF) (38–41). In the case of HTLV-I and -II, Tax appears to interact with three 21-base pair (bp) repeats located in the U3 portion of the viral LTR. Within the sequence of the 21-bp repeats are core sequences known to bind cellular transcription factors (42,43). A variety of these proteins have now been identified and

partially characterized.

In contrast to the HTLV promoters, Tax activation of the IL-2Rα gene involves induced nuclear expression of a cellular DNA binding protein, which interacts with an NF-κB-like enhancer (44). NF-κB is a DNA-binding factor first shown to interact with the enhancer of the κ light chain immunoglobulin gene (45). Tax interaction with NF-κB is thought to account for inducibility of some other cellular and viral promoters such as the HIV-1 promoter. The lack of an NF-κB-like binding site in the HTLV-I/-II promoter and deletion of NF-κB-like binding sites from the GM-CSF promoter with retention of response to Tax indicate that Tax may act via different pathways in different cellular and promoter contexts (41).

To date, only limited evidence directly implicates Tax in T-cell transformation. Introduction of Tax coding sequences under the control of a herpes saimiri vector has resulted in continuously proliferating T-cell lines *in vitro*, although the transformed cell lines appear to retain dependence on IL-2 for continued growth (46). Expression of HTLV-I Tax under the control of the HTLV-I LTR in transgenic mice does not lead to Tax expression in T-cells, and T-cell malignancy is not observed (47). Some mice developed mesenchymal tumors reminiscent of neurofibromatosis, as well as muscular atrophy. Recent HTLV-I Tax transgenics under control of the T-cell-specific Thy-1 promoter also did not result in T-cell malignancy (48). Nevertheless, the promiscuous interaction of Tax with a variety of viral and cellular promoters suggests that it may play a pivotal role not only in the HTLV-I life-cycle, but also in definition of the malignant phenotype. Tax can *trans*-activate the IL-2Rα gene, which offered an explanation for the high degree of Tac (high affinity IL-2 receptor) antigen expression in HTLV-I-transformed T-cells. Similarly, ectopic GM-CSF production due to Tax may cause eosinophilia, which is frequently seen in ATLL. In addition, Tax may also be involved in *trans*-activation of the parathyroid hormone-related protein (PTHRP) promoter, perhaps accounting for the ectopic expression of PTHRP in ATLL cells, thereby leading to altered calcium metabolism (49). However, HTLV-I mRNA expression in ATLL is so low that it has required use of RNA polymerase chain reaction (PCR) to be detected. Therefore, whether effects seen with Tax *in vitro* have applicability to HTLV-I *in vivo* remains unclear.

The ability of the viral *trans*-activator, Tax, to interface with several cellular transcriptional factor pathways suggests a new model for viral leukemogenesis. The presence of such *trans*-acting genes may allow development of new assays for the presence of as yet undiscovered retroviruses based on the ability of *trans*-acting 'Tax-like' transcriptional regulatory proteins to act on cellular and viral genes. Models for cooperation between oncogenes could undoubtedly be applied to help dissect a potential role for Tax in cooperation with other oncogenes. New models for oncogenic cooperation have emerged at this conference, such as superinfection of E_μ-*myc* transgenic mice with Moloney murine leukemia virus (MoMuLV) (50,51).

Several conclusions can be derived from study of the *trans*-regulatory *tax* gene: (a) new mechanisms of retroviral leukemogenesis other than transduction of cellular proto-oncogenes and/or retroviral insertion adjacent to cellular proto-oncogenes may be operative in human malignancy; (b) human retroviruses possess transcriptional activators that may affect expression of cellular genes, and aberrant expression of cellular genes may contribute either to leukemogenesis *per se*, or to the leukemic phenotype; and (c) the effect of viral transregulatory genes may be felt early in leukemogenesis, and may be insufficient to elicit the full-blown leukemic phenotype.

An additional transregulatory gene studied more recently is the *rex* gene of HTLV-I, -II, and BLV. The *rex* gene is required for productive HTLV-I/-II infection. The *rex* gene of HTLV-I encodes two proteins, one of 27 kDa and one of 21 kDa, from an overlapping reading frame to that encoding $p40^{tax}$ (52). These proteins appear to result from utilization of an alternative initiator methionine. In HTLV-II, two proteins are also encoded of the apparent sizes, 26 and 24-kDa (53). In HTLV-II, these appear to derive from different degrees of phosphorylation, with the larger molecular weight species being a hyperphosphorylated form of the 24-kDa protein (54). In both HTLV-I and -II, the proteins appear to act as post-transcriptional regulators, and elicit export of full-length *gag/pol* mRNA and probably partially spliced *env* transcripts from cell nucleus to cytoplasm. The *rex* gene appears necessary to allow expression of non-spliced and partially spliced viral mRNA, which in turn allows synthesis of Env and Gag proteins and production of mature virions.

In HTLV-I, Rex has been found to act through a *cis*-acting Rex-responsive element (RxRE) located in the 3' LTR. Our group has studied Rex effects mediated through the 5' LTR of HTLV-II (55). In both cases, Rex appears to act through sequences located in the R region, downstream from the transcription initiation site. Assays of binding to radiolabeled viral RNAs have demonstrated that purified HTLV-II Rex can directly bind to transcripts initiated from the 5' LTR, and that binding occurs to a portion of a *cis*-acting element responsible for Rex action, known as the RxRE (56,57). Mapping in our laboratory has demonstrated that Rex can bind directly to transcripts as short as 115 bp derived solely from sequences within the R region (57,58). These transcripts contain the 5' LTR splice donor site, and mutation of the splice donor site appears to impair Rex binding and function. Furthermore, Rex binding is dependent on retention of a specific stem-loop mRNA structure located downstream from the splice donor site (from nucleotide 465–501 within the HTLV-II 5' LTR) (57). This stem-loop structure is conserved in both HTLV-I and -II. Rex binding may be facilitated by hyperphosphorylation, and it would appear to be the higher molecular weight (26 kDa) Rex species of HTLV-II that binds efficiently, indicating that cellular controls on Rex function may exist at the level of phosphorylation

(Chen and Green, unpublished observations). Nucleolar localization and our results using RxRE mutations of the splice donor site suggest a direct interaction of Rex with the cellular splicing apparatus to facilitate bypass of cellular splicing mechanisms.

An intriguing observation regarding Rex of HTLV-I was first made by Rimsky and Greene, demonstrating that HTLV-I Rex can functionally substitute for the Rev protein of HIV-1 (59). The Rev protein of HIV-1 performs an analogous function to that described for Rex in HTLV-I. Their assay demonstrated the capacity of Rex to induce production of the truncated single-exon form of the HIV-1 Tat protein that reflects translation from unspliced *env* vector mRNA (57). HTLV-II Rex in our laboratory is also able to rescue replication of Rev-deficient mutants of HIV-1 (58). Rescue of HIV-1 Rev-deficient mutants by HTLV-II Rex is relatively inefficient, and this can partially be accounted for by the relatively low affinity of HTLV-II Rex binding to the HIV-1 Rev-responsive element (RRE) (58). In addition, HIV-1 Rev is unable to complement an HTLV-II Rex-deficient clone, indicating a non-reciprocal pattern of complementation. Nevertheless, the ability of Rex to complement the genetically distant HIV-1 virus in *trans* suggests that, like Tax, Rex may act promiscuously on a variety of non-HTLV target sequences. This raises the possibility that Rex may also interact with cellular RNA to elicit aberrant splicing and/or transport. Disrupted processing and expression of cellular mRNAs could conceivably be implicated in the process of leukemogenesis as well. Therefore, post-transcriptional regulators may also be involved in the process of retroviral leukemogenesis. Direct evidence supporting this hypothesis has not been obtained.

Disparate Disease Entities Related to HTLV-I

Approximately five years following its discovery, it was found that the pathology elicited by HTLV-I in one setting may not predict other forms of pathology related to the virus. A specific illustration is afforded by the two major non-overlapping syndromes associated with HTLV-I. While HTLV-I may cause ATLL, another subset of infected individuals, approximately half as many, will develop a slow neurologic disease characterized by gradual development of spastic paraparesis of the lower extremities with minimal sensory loss. This illness, tropical spastic paraparesis (TSP) (also known as HTLV-I-associated myelopathy (HAM)), is distinguished from multiple sclerosis (MS) by virtue of its chronic progressive and non-episodic nature, as well as the general limitation of pathology to motor control in the lower extremities and sphincter dysfunction. The association between this illness and the virus was discovered by Gessain and co-workers while screening neurologic illnesses for retroviral involvement in Martinique (60). As opposed to the leukemia, where HTLV-I has been observed to infect and transform T-cells *in vitro*, no adequate model for pathogenesis of the myelopathy exists. Regardless of underlying mechanisms, involvement of HTLV-I in a slow neurologic disease was not predictable on the basis of its involvement in T-cell leukemia. The latency period for development of HAM is also appreciably shorter, and recently at UCLA, we saw a patient develop myelopathy approximately fifteen months following infection by transfusion (61). In contrast, development of ATLL following transfusion-acquired HTLV-I is almost never seen. Furthermore, co-existent ATLL and HAM have rarely been described. We have observed at least one case of multiple members of an Iranian Jewish family developing HAM (D. Meytes *et al.*, unpublished), and this has been reported by other investigators. This would suggest that either differences in host genetic make-up and susceptibility or differences in viral isolates may account for familial HAM. It is important to note that the association between HAM and HTLV-I was made serendipitously. Quite possibly, if the link to HAM had been described first, no search for HTLV-I association with malignancy would have been made. This would suggest that other viruses that may not be associated in investigators' minds with development of malignancy may be candidates for potential oncogenic roles. Good candidates would be viruses with *trans*-acting transcriptional proteins, such as members of the herpes family, adenovirus, and/or other retroviruses.

DISCUSSION

Over a decade following initial identification of the role of the retrovirus in human disease, HTLV-I remains as the only example for human retroviral leukemogenesis. Several lessons pertinent to the search for leukemogenic viruses can be derived from the HTLV model. First, leukemia may be the rare consequence of infection with a relatively common virus. Second, a long latency period may be involved following acquisition of infection, and the leukemogenic process may involve multiple steps beyond initial infection. Third, the mechanism by which the virus may predispose to development of leukemia may be different from that seen with other retroviruses, and may involve *trans*-acting viral genes, either regulators of DNA transcription or regulators of viral mRNA expression. Finally, the role of HTLV-I as an etiologic agent in both HAM and ATLL suggests that viruses not thought to have oncogenic properties may on occasion predispose to malignancy. The overall lesson from the HTLV experience is that when suitable or suggestive epidemiologic factors are found, such as ethnic, geographic and/or familial clustering in the absence of a known inheritance pattern, a search for underlying viruses should be initiated. The search would involve removal of malignant tissue, when possible, for culture *in vitro* and a search using available molecular and serologic probes. The success in identifying HTLV-II by virtue of limited nucleic acid homology and antigenic similarity to HTLV-I suggests that new retroviruses can be identified. The demonstrated crossreactivity between HTLV-I and -II suggests that any search should be accompanied by rapid isolation of nucleic acid probes for viral sequences of interest, so that crossreactive

entitites can be discerned. A fresh look should be taken using newly available probes as a means of determining viral clonality, particularly for DNA viruses such as herpesviruses to assess whether a particular malignant tissue has arisen from a single virally infected cell. Furthermore, scrutiny of viruses already known to be widespread in the population may prove fruitful, as already appears to be the case for EBV and a subset of Hodgkin's disease. A re-duplication of such efforts will determine whether new retroviruses with oncogenic potential will be identified in man in the upcoming decade, or whether HTLV will remain an isolated if fascinating example of retroviral leukemogenesis in man.

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Immunoglobulin Prophylaxis against HTLV-I in a Rabbit Model

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We have investigated the protective effect of human T-cell leukemia virus I (HTLV-I) immune globulin (HTLVIG) against HTLV-I in rabbits. HTLVIG containing 77 mg/ml of IgG was prepared from pooled plasma from seropositive healthy persons. In the first experiment, four groups (A, B, C, and D) of three rabbits were transfused with 5 ml blood from an HTLV-I-infected rabbit. Groups A, B, and C were infused 24 h later with 10, 5, and 2 ml HTLVIG, respectively, while group D was infused with 10 ml HTLVIG 48 h later. Seroconversion for HTLV-I occurred in none of group A, one of group B, and all of groups C and D after 2–5 weeks. In the second experiment, four litters (E, F, G, and H) born to another virus-infected rabbit and consisting of 7, 5, 7, and 7 newborns, respectively, were used. Litters E and H were allowed to grow normally as controls, while litters F and G were given intraperitoneal inoculation of 3 ml/kg of HTLVIG weekly four times until weaning. Although three of litters E and H each seroconverted after 5–8 weeks, none of litters F, and one of litter G became antibody-positive after 10 weeks. Presence or absence of HTLV-I infection in all these animals was confirmed by transfusion assay or gene amplification. These results indicate that passive immunization protects rabbits against blood- and milk-borne transmission of HTLV-I.

INTRODUCTION

A rabbit model of human T-cell leukemia virus I (HTLV-I) infection has been established, in which the virus was shown to be transmissible not only by blood transfusion (1,2) but also from dam to offspring via milk (3,4). In the blood transfusion experiment, as little as 0.01 ml blood from a virus-infected rabbit was capable of transmitting HTLV-I (2). Furthermore, milk or semen lymphocytes from seropositive healthy persons transmitted HTLV-I when inoculated intravenously into rabbits (5). This animal model, therefore, provided a unique opportunity to study the protective effect of passive immunization against HTLV-I (2,6). In the present experiment, immunoglobulin prophylaxis against blood- and milk-borne transmission of HTLV-I was further explored.

MATERIALS AND METHODS

Rabbits

Japanese white rabbits, weighing about 3 kg, purchased from a commercial breeder were used.

Detection of Antibodies to HTLV-I

Blood samples were taken from rabbits at intervals of 1–2 weeks and sera were titrated for HTLV-I antibodies by indirect immunofluorescence against the MT-2 cell line as described previously (2). The presence or absence of immunoglobulin G (IgG) antibodies was verified by Western blot using a MT-2 lysate as antigen. Sera were also tested for IgG and immunoglobulin M (IgM) antibodies by enzyme-linked immunosorbent assay (ELISA) against disrupted HTLV-I virions according to the manufacturer's instructions (Eisai, Tokyo). Neutralizing antibodies were assayed against vesicular stomatitis virus (VSV) bearing envelope antigens of HTLV-I as previously described (7).

HTLV-I Immune Globulin (HTLVIG)

HTLVIG containing 77 mg/ml of IgG was prepared from pooled plasma from seropositive healthy persons by the method of polyethylene glycol fractionation (8). The preparation had an immunofluorescence anti-HTLV-I titer of 1:5120 and a VSV (HTLV-I) pseudotype neutralizing antibody titer of 1:6250.

Transfusion Assay

To ascertain the status of HTLV-I infection, 20 ml of blood obtained from experimental rabbits were transfused into normal rabbits. Seroconversion of the recipient rabbits indicated a virus carrier state of the donor rabbits.

Polymerase Chain Reaction (PCR)

DNA extracted from peripheral blood mononuclear cells was analyzed for the presence of HTLV-I sequences by the method of Kwok *et al.* (9). DNA, 1 μg, was subjected to 40 cycles of denaturation followed by annealing and extension. Oligonucleotide primers at 7341–7360 and 7460–7411 corresponding to the pX region of HTLV-I were used. Amplification was performed using a thermostable DNA polymerase on an automated DNA Thermal Cycler (Perkin-Elmer/Cetus, Norwalk, CT). The amplified products were electrophoresed on 6% polyacrylamide gels, transferred to nylon membranes, and hybridized with a ^{32}P end-labeled probe at 7364–7383.

RESULTS

Passive Immunization against Blood-borne Transmission of HTLV-I

Four groups (A, B, C, and D) of three rabbits were first transfused with 5 ml of blood from an HTLV-I-infected rabbit. Groups A, B, and C were infused 24 h later with 10, 5, and 2 ml HTLVIG, respectively, while group D was infused with 10 ml HTLVIG 48 h later.

Seroconversion for HTLV-I occurred in none of group A, one of group B, and all of groups C and D after 2–5 weeks (Figure 1). All five rabbits which were protected from seroconversion remained seronegative during an observation of six months. Sera taken immediately after infusion of HTLVIG showed anti-HTLV-I titers of 1:320 for groups A and D, 1:80 for group B, and 1:20 for group C. The VSV (HTLV-I) pseudotype neutralizing titers of these sera were 1:1250

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LEUKEMIA

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Serological and molecular survey for HTLV-I infection in a high-risk Middle Eastern group

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To define the extent of human T-cell leukaemia virus (HTLV-I) infection among a group of Jewish immigrants to Israel with an increased frequency of adult T-cell leukaemia, various serological and molecular screening methods, including enzyme-linked immunosorbent assay (ELISA) for anti-HTLV-I, ELISA for antibody to recombinant HTLV-I p40*tax* protein, and molecular detection of infection by polymerase chain reaction (PCR) amplification of HTLV-I proviral DNA from peripheral blood mononuclear cell DNA, were used. By HTLV-I ELISA the overall rate of infection was 12% (24 of 208) among immigrants from Khurusan, northeastern Iran; no HTLV-I carriers were detected among 111 unselected Jewish immigrants from other parts of Iran. There was unexplained clustering of HTLV-I infection within a cohort of 32 elderly women of similar geographic origin in a home for old people—14 were seropositive by ELISA and 19 of 29 were positive by PCR. The findings in this newly identified high-risk population suggest that in addition to ELISA, other screening techniques may be required to detect all carriers in high-risk populations.

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Introduction

Human T-cell leukaemia virus type I (HTLV-I) infection has been described in southern Japan, the Caribbean basin, and the northern parts of South America, and in certain high-risk groups, such as intravenous drug abusers in the United States.[1,2] Previous reports of HTLV-I infection among Ethiopian Jews in Israel were not confirmed.[3,4] During the past 4 years, sporadic cases of adult T-cell leukaemia linked to HTLV-I have been reported in Israel[5,6] and 1 of the 5 latest cases were among immigrants to Israel who originated from the city of Mashad in northeastern Iran.[6] Because of these findings, we undertook a systematic survey of Iranian Jews in Israel, focusing on immigrants with links to Mashad.

Subjects and methods

Blood samples from Israeli blood donors of Iranian origin were obtained from the Israeli Magen David Adom Blood Services Center, Tel Aviv. The criterion for classification as an Iranian control was that the country of birth of the blood donor or at least one of his or her parents was Iran. Blood samples were collected on three occasions from residents of a Mashadi home for elderly women in the Tel Aviv area and from three Mashadi community synagogues in the cities of Bnei Brak and Tel Aviv. Samples were classified as Mashadi if the donor or at least one of his or her parents originated from Mashad, Iran. 20 samples from patients on long-term haemodialysis, 8 from patients with T-cell malignant disorders other than adult T-cell leukaemia, and 12 from Ethiopian Jewish immigrants were also included.

Serological screening was done for HTLV-I antibodies on serum or plasma samples by means of an enzyme-linked immunosorbent assay (ELISA; Abbott Laboratories). Confirmatory western blotting and or radioimmunoprecipitation assay (RIPA) with sulphur-35-labelled methionine HTLV-I-infected HUT 102B

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PREVALENCE OF HTLV-I INFECTION

	HTLV-I ELISA and western blot		Anti-p40*tax* ELISA	
	No tested	No (%) positive	No tested	No (%) positive
Mashadi Jews	208	24 (12%)	127	12 (9%)
Other Iranian Jews	111	0	20	0
Ethiopian Jews	12	0	12	0
Haemodialysis patients	20	0	20	0
Patients with T-cell malignant disorders	8	0	ND	

lysate were also done.[11] Samples positive in the ELISA were tested by both confirmatory methods. Antibodies to HTLV-I p40*tax* were measured by means of an ELISA with recombinant p40*tax* as antigen on the solid phase (polystyrene beads) (Abbott). HTLV-I seropositive infected samples with known reactivity against p40*tax* on RIPA were used as positive controls, and 8 samples negative for HTLV-I on ELISA and western blot as negative controls. Samples were scored as positive for p40*tax* if the optical density exceeded 4·5 times the mean negative control value. The polymerase chain reaction (PCR) was used to amplify HTLV-I sequences of DNA from peripheral blood mononuclear cells with primers to a 159 bp segment contained within the *tax/rex* gene as previously described.[12]

Results

The extensive social and ethnic ties between Jews of Mashadi origin allowed selective sampling of this population within Israel. On repeated assays, 24 of 208 (11·5%) Mashadi serum samples were positive by ELISA for antibodies to HTLV-I and were confirmed by western blotting. In contrast, none of the 151 control samples was positive by ELISA (table).

In an effort to survey the oldest members of the Mashadi population, we tested 32 unrelated elderly Mashadi women living in an old people's home in Tel Aviv. 14 (44%) were seropositive, a rate three times higher than that in the general Mashadi population. At our first sample collection in 1988, 12 (52%) of 23 long-standing residents of the home were seropositive; 2 of 9 new residents surveyed a year later were seropositive. Of 26 Mashadi women older than 60 years who lived elsewhere in Israel only 3 (12%) were seropositive.

To determine whether serological assays had detected all infected subjects, we carried out PCR amplification of HTLV-I DNA from 29 of the residents of the old people's home. 19 were positive for HTLV-I sequences: 14 of these were seropositive, 4 were seronegative, and 1 was seronegative by standard ELISA but reactive in the anti-p40*tax* ELISA. A second PCR on repeat samples from 10 of these women showed concordant results in 6 of 6 PCR-positive and 2 of 3 PCR-negative cases; 1 woman originally positive on PCR was negative on the second sample, and 1 woman originally PCR-negative was faintly positive on her second sample and had antibodies to p40*tax* by ELISA. This unusual clustering of HTLV-I infection within the old people's home suggested acquisition of infection within the institution.

In addition to antibodies to virion components, we assayed for antibodies to the non-structural p40*tax* protein. Of 128 Mashadi samples tested 12 had absorbance levels 4·5 or more times those of the negative control and were judged positive (table). 103 samples were negative by both assays. 8 samples were seropositive for both anti-HTLV-I and anti-p40*tax*; 13 samples were positive for anti-HTLV-I and negative for anti-p40*tax*; and 4 were positive for anti-p40*tax* but repeatedly negative for anti-HTLV-I by ELISA and western blot. 3 of the latter group of samples were negative on PCR, and the other, from a resident of the old people's home, was positive. All 4 were negative by western blotting and by RIPA. Therefore, the reason for the high titres in three samples in the anti-p40*tax* ELISA is unclear.

Direct comparison of anti-p40*tax* ELISA results and those of the RIPA was done for 28 subjects. 8 samples were positive for anti-p40*tax* by both RIPA and ELISA; 1 with traces of antibody by RIPA was negative by ELISA; the 3 samples mentioned previously were negative by RIPA but repeatedly positive by ELISA; and 16 were negative by both tests. The usefulness of the anti-p40*tax* ELISA in detecting true additional HTLV-I-infected seronegative individuals remains unclear, since seronegative infection was corroborated by other means of detection in only 1 of the 4 questionable cases.

The finding of a higher rate of seropositivity among elderly Mashadi women than in the general Mashadi population suggested a potential for gradual development of seropositivity with age, as has been reported by others. Because of the possibility that serological screening may not detect all positive subjects' we carried out HTLV-I-specific PCR on DNA from 68 randomly selected Mashadi blood samples. 5 of 7 known seropositive samples were positive in the PCR assay, and 61 seronegative samples were all negative by PCR. Hence, PCR did not increase sensitivity of detection within this sample above that achieved with serological assays alone. All PCR-positive samples contained HTLV-I and not HTLV-II, as shown by discriminatory PCR (data not shown).

Discussion

We have identified a high risk of HTLV-I infection in Iranian Jews originating from the city of Mashad in Khurusan, northeastern Iran. This group seems to have an overall infection rate of about 12%, though the rate was much higher among residents of a Mashadi home for old people. The reason for this clustering is unclear, although the findings suggest acquisition of infection within the institution. The overall level of infection among other Iranian Jews seems to be substantially lower than that among Mashadis. The explanation may be geographic, and Mashad may be within a previously unrecognised endemic region. Since we did not test non-Jewish Mashadis, this possibility cannot be excluded.

The unique history of the Mashadi community may help to explain their high rate of HTLV-I infection. In 1839, the Jewish community in Mashad was subjected to a series of violent attacks and forced to convert to Islam, though the majority of the community continued to practise Judaism covertly.[13] To safeguard the community secret necessitated a very high rate of intermarriage among community members. Members of this community continued to marry close relatives over the next 150 years. Markers of consanguinity are high among Mashadi Jews—for example, over 20% have a deficiency of glucose-6-phosphate dehydrogenase (D. M., unpublished). The chance introduction of HTLV-I infection into the community could have resulted in rapid transmission of the virus by sexual means and by breastfeeding.

An estimated 5000–6000 Mashadis now live in Israel. This is the first Israeli ethnic group identified as having a high rate of HTLV-I infection. Members of the Mashadi Jewish community also migrated to the USA and parts of

Europe. We predict that the rate of infection among these migrants would be similar to that of the Israeli cohort.

In this study, we used several methods to detect HTLV-I infection. Our rate of anti-p40*tax* seropositivity in carriers was somewhat lower than that found by others among healthy HTLV-I carriers.[14,16] Serum samples from 4 subjects were positive only for anti-p40*tax* antibodies. Independent evidence of infection was obtained by PCR in only 1 of the 4. Thus, detection of anti-p40*tax* antibodies did not appreciably add to the estimate of the rate of infection. Our findings on the use of PCR suggested that in a high-risk population, such as the old people's home we studied or in families of HTLV-I carriers, PCR would increase the number of infected individuals above that detected by serological means. The usefulness of PCR as a screening assay in appropriate settings requires further study.

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Quinine-induced disseminated intravascular coagulation

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Recurrent disseminated intravascular coagulation occurred in 3 women after ingestion of quinine tablets for cramp. All had circulating quinine-dependent antibodies to platelets and in 2 there was initial evidence of antibody consumption, with low titres that rose steeply over the next few days and remained high for many months.

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Introduction

Recognised haematological problems associated with ingestion of quinine include thrombocytopenia, erythrocyte haemolysis, and neutropenia. Quinine was first implicated as a cause of purpura in the late 19th century,[1] and there have been several reports of associated thrombocytopenia.[2,3] However, we are aware of only two published cases of disseminated intravascular coagulation induced by quinine,[4,5] and report three further cases.

Patients and methods

Case histories

A 71-year-old woman was admitted 5 times over 3 years with various symptoms, which included acute shortness of breath, wheeze, generalised abdominal pain, fever, lower back and chest pain, melaena, haematemesis or haemoptysis, and bruising and petechiae. Most episodes occurred shortly after going to bed. Investigations on each occasion (table) showed evidence of disseminated intravascular coagulation (DIC). On the first 2 admissions she was treated with antibiotics, although blood cultures were always negative. On the third admission she was treated for asthma, and on the last 2 occasions no specific treatment was given. On each occasion, fever and other symptoms resolved within 24 h with a subsequent resolution of coagulation abnormalities. At least 2 similar, but milder, episodes also occurred for which she did not attend hospital. On 3 occasions recent quinine ingestion was clearly remembered by the patient or documented in the admission notes. A check on the number of tablets left in the bottle after her initial prescription indicated that 10 tablets had been taken over 7 years. Retrospective quinine-dependent platelet antibody analysis on samples stored from the last 3 admissions and from intervening periods showed low or undetectable antibody concentrations during the first 2 days of each acute episode, which then rose sharply

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MOLECULAR CHARACTERIZATION OF HTLV-I INFECTION IN ISRAEL

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Short Title: Characterization of Israeli HTLV-I isolate

INTRODUCTION

Many studies were performed on human T-cell leukemia virus type I, ethiologically associated with adult T-cell leukemia (ATL) [1,2] and HTLV-associated myelopathy (HAM).[3-7]

HTLV-I genomes isolated from ATL and HAM patients from different geographical origin demonstrated a high degree of homology (>96%).[5-8] Sequence variation in different isolates were found mostly in the LTR (1.3-5.2%) and the region between the envelope and tax/rex reading frames (0.1 6.9%).[6] It was found that genetic diversity between different isolates is in association with the geographical origin and not with the clinical presentation.[6,7,8] In 1988, HTLV-I was first discovered in Israel and the Middle East.[9] Two years later a community of jewish immigrants from the city of Mashad in northeastern Iran was identified with an infection rate of about 12%.[10]

Our aim was to determine the nucleotide sequence of LTR and env gene from HTLV-I genome of an HAM patient who originated from Mashad. This data was compared to sequences derived from HTLV-I isolated from Japanese and African patients in order to locate the origin of the Mashadi virus.

MATERIALS AND METHODS

Cell lines: Lymphocytes were collected from 20ml peripheral blood by a Ficoll-Hypaque density gradient and were resuspended in RPMI-1640

medium supplemented with 15% fetal bovine serum, interleukin-2 (IL-2) at a concentration of 50u/ml, 0.5% PHA and 1% penicillin/streptomycin. Cells were incubated at 37^{o}C in the presence of 5% CO2 and were maintained for several weeks. The cells were cryopreserved at various intervals for DNA extraction.

DNA extraction and PCR amplification: DNA was extracted from lymphocytes with phenol/chloroform. Two regions in the HTLV-I genome were amplified: LTR and env gene. LTR amplification was performed with the primers R11/14 which defined a 741 base sequence from nucleotide 61 to 802 (nucleotides are numbered according to the sequence of Seiki et al.[11] The env gene was amplified by two pairs of primers : R15/17A which defined a 1163 base sequence from nucleotide 5201 to 6364 and R19/18 primers which defined 791 base sequence from nucleotide 5942 to 6733.

The patient's (HE) DNA was PCR amplified for 30 cycles in a reaction containing 100 μl of 2mM MgCl, 200 ng from each primer, 0.2 mM from each dNTPs and 2.5 u of taq polymerase (USB, Cleveland, OH).
A list of primers used for PCR amplification is presented in table 1.

DNA sequencing: PCR products were recovered from PCR reaction with DS primer Remover (Advanced Genetic Technologies Corp, Gaithersburg, MD) and ethanol precipitation. Nucleotide sequence analysis was performed by the Taq Dye Deoxy Terminator Cycle Sequencing kit using the 373A DNA Sequencer (Applied Biosystems) at the Biological Services of the Weizmann Institute Rehovot Israel. Sequence analysis was performed at least twice for each primer (Table 1).

Sequence comparison: HE sequence was compared to the Japanese (ATK), African (EL) and Papua New Guinea (PNG-1) sequences by gcg program with the accession numbers: *JO2029* (ATK), *S74562* (EL LTR), *M85207* (PNG-1) and M69044 (EL).

RESULTS

Comparison of HTLV-I (HE) LTR to other isolates. PCR amplification of HTLV-I LTR produced a PCR product of 741bp of which 698 bases from it were sequenced. 7 nucleotides which are not verified yet are designated as N. HTLV-I-(HE) LTR sequence was compared to three other isolates. The Japanese (ATK) sequence showed nucleotide homology of 97.8% with 15 nucleotide differences.[11] The African sequence showed nucleotide homology of 95% with 35 nucleotide differences and the Papua New Guinea isolate, which was compared only by 629 nucleotides, showed 91% homology with 56 nucleotides differences.[5,6]

Comparison of the sequence changes in the LTR region to the Japanese sequence showed that the differences are clustered mostly in the U3 (66.7%), 6.7% were in the R and 26.7% were in the U5 regions. There are no changes at the three 20bp enhancer elements at the U3 region and there is 98.7% homology for the Rex Responsive element located between bases 313 and 627.

Comparison of the env gene amplified from HTLV-I (HE) genome to the Japanese and African sequences. The PCR product was 1532bp in length from which 1496 bases were sequenced. The sequence between nucleotides 5625-5685 is not verified yet.

Comparison to the Japanese (ATK) sequence showed nucleotide homology of 97.7% with 9 nucleotide differences while the African (EL) sequence showed nucleotide homology of 98.4% with 6 nucleotide differences.[12,14] There is a problem with base T at position 5400. It seems that it creates a stop codon while in the other two sequences it does not exist. This finding needs further examination. HE env2 sequence was compared to the Japanese (ATK) sequence.[11] 98.7% homology at the nucleotide level with 12 nucleotide differences were found. Comparison to the African (EL) sequence showed nucleotide homology of 99.5% with 5 nucleotide differences.[12]

DISCUSSION

A new focus of HTLV-I infection was recently identified in the Middle East.[10] In this study we determined the sequence of two regions in HTLV-I isolated in Israel from an HAM patient belonging to this community: the LTR region and the env gene. Several studies have indicated a high degree of homology among HTLV-I isolates (>96%) and demonstrated that differences between variants are in association with their geographical origin.[5,6,7,8] Comparison of Mideastern sequence which originates in Iran to the Japanese, African and Papua New Guinea sequences in order to examine whether there is indeed a higher degree of homology between isolates from the same geographical area. A high level of homology, at about 98%, to the Japanese isolate, while the African and Papua New Guinea isolates were more divergent with sequence homology of 95% and 91%, respectively.

Comparison of HTLV-I env gene to the Japanese and African isolates showed homology at the nucleotide level of about 98-99%. From these results we can conclude that the env gene is more conserved than the LTR region.

With the exeptions of some nucleotides which are not verified yet, comparison of the LTR region showed a higher degree of homology between Iranian sequence and the Japanese isolate. As for the env gene, there is a high degree of homology between the Iranian sequence and the African isolate. With these results we cannot conclude if there is an influence of the geographical area on the virus genome.

There are two theories about HTLV-I origin and the way it was spread to the world. Gallo suggests that it originated in Africa and spread to the new world and Japan, while Saskena et al. suggest that HTLV-I originated in the Indo-Malay region.[5,13,14] Based on the comparison of the LTR sequences, our results support the theory of HTLV-I originating in Africa, since we found higher degree of homology between HE sequence and the African isolate, than with the sequence of Papua New Guinea isolate.

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Table 1: LIST OF PRIMERS FOR PCR AMPLIFICATION AND SEQUENCING

Primer	From nt.	to nt.	Used for	Seq.	Remarks
R11	61	77	amp. & Seq.	5' TAGAGCCTCCCAGTGAA	
R12	494	470	seq.	5' CCTAGACGGCGGACGCAG	Comp.
R14	802	786	amp. & seq	5' CTCGTATCCCGGACGAG	Comp.
R15	5201	5218	amp. & seq.	5' CATGGGTAAGTTTCTCGC	
R16	5660	5645	seq.	5' ATGGAGATTAATATTG	Comp.
R17	5641	5658	seq.	5' GCCTCAATATTAATCTCC	Comp.
R19	5942	5959	amp. & seq.	5' TCCATCCTCTTCTTCTAC	
R17A	6364	6347	amp.	5' TCCCAGAACAGGAGATCA	Comp.
R18	6733	6716	amp. & seq.	5' GGGAGAGGTAATTATTG	

Editorial

T-LYMPHOCYTES IN CHILDHOOD LEUKEMIA

Yehuda L. Danon, MD □ Kipper Institute of Immunology, Chilren's Medical Center of Israel, Tel-Aviv University, Sackler School of Medicine, Kaplan Street 14, Petah-Tikva 49202, Israel

□ The recent decade is associated with major breakthroughs in lymphocyte immunotyping. In view of the excellent improvement in chemotherapy results in acute lymphoblastic leukemia (ALL) and the chemotherapy associated mortality, Dr. Lovat and associates studied serially the T-lymphocytes in childhood leukemia[1].

The immunophenotyping classification of ALL is part of the diagnostic profile that includes morphology cytochemistry and genetic karyotypic assay.

Immunophenotypic classification divides ALL to common ALL with blasts expressing HLA-DR, CD-19 and CD-10; null ALL with HLA/DR and/or CD-19 antigens presenting on cell surfaces; and pre B-ALL with immunophenotyping profile including HLA-DR, CD-19 and CD-10 with cytoplasmic μ-chain[2-3].

The serial study of T-lymphocyte subpopulation quantity and function published in this journal[1] reveals a significant and marked decrease in circulating T helper cells CD4—number and significant but less profound fall in CD8 Cytotoxic T cells compared to normal controls.

Polyclonal and specific HSV-1 proliferation responses showed a slightly but significantly decreased response in cALL patients; however, marked impairment of T-cell response to specific recall antigens or polyclonal stimulation is not demonstrated. Although there were persistent reports of heterogeneity of the helper T-cell compartment, only relatively recent were distinct types of helper T cells resolved defined primarily by the different patterns of lymphokine synthesis that became also a convenient marker to describe T help cells subclass differences, but an extensive study of IL-2 and IL-4 production could not show any difference from control patients.

A recent study, aimed at investigating whether CD4+ T cells are predetermined to produce given patterns of lymphokynes, showed that IL-2-producing clones can be derived from the same cells as IL-4-producing clones,

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supporting the view that the IL-2-producing Th1 or the IL-4-producing Th2 phenotype of a T cell clone is acquired during T cell differentiation[3].

Differential production of cytokines by helper T cells during the immune reaction has important regulatory effects on the nature of the response and induction of Th1, and because Th2 responses play a key role in the natural response, it seems that availability of additional monoclonal antibodies, improved immunophenotyping methods and better understanding of cytokine mechanisms will improve our monitoring capabilities in the future. It was shown that CD8+ T cells may switch the response induced by antigens and antigen-presenting cells from humoral to a cell mediated role.

Lymphocyte phenotyping was assessed by Lovat et al.[1] using indirect immunofluorescence on blood smears. The availability of a variety of monoclonal antibodies dramatically increased our possibilities to study cellular subpopulations. Microscopic quantitation methods of immunofluorescence are ill suited to accurately count large numbers of cells; direct or indirect immunofluorescence is more accurately analyzed by flow cytrometry with the possibility of sorting and functually studying target populations. Even with flow cytrometry, the differences in instrumentation, antibodies and techniques may introduce significant sources of variation.

Infection is a major cause of morbidity and mortality in leukemic patients. Infections are bacterial, protozoal, viral and fungal, although the underlying mechanism for the increased susceptibility to infection is not clearly defined. The present work widens our knowledge and scope of the prediction of susceptible populations. It seems that a wide, long-range prospective study is needed defining predictors of infections, possible modifications needed in chemotherapy and other ways to treat immunosuppression.

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Annotation

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FOREWORD

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PI Signature _____ July 10th, 1993 Date

MOLECULAR STUDIES OF HTLV-I INFECTION IN NEWLY RECOGNIZED HIGH RISK POPULATION - MIDTERM REPORT

TABLE OF CONTENTS

INTRODUCTION:

Human retroviruses have been recognized and well characterized during the last decade after the detection of human T-cell leukemia virus type I (HTLV-I) by Poiesz and Gallo (1) and Yoshida (2) endemic areas were described in southern Japan and parts of the Caribbean and South America (3-7), as well as high risk groups of IVDA (Intravenous Drug Abusers) (8). Following the discovery of human immunodeficiency virus types 1 (HIV-1) and 2 (HIV-2) and their implication in AIDS accelerated the intensity of research for possible oncogenic viruses. HTLV-I is the human retrovirus known as the cause or ethiologic agent of Adult T-cell Leukemia (ATLC) and of the chronic progressive demyelinating process HTLV-I-associated Myelopathy HAM, known also as Tropical Spastic Paraparesis - TSP.

It was found that leukemic cells in malignancies associated with HTLV-I contained clonically integrated provirus, leading to the direct viral involvement in the oncogenic process, while in contrast, neoplasms frequently seen in the setting of HIV infection - (Kaposis Sarcoma and high grade B-cell Lymphoma) did not appear to arise as a direct consequence of viral transformation of HIV-1 infected cells. HTLV-I studies were not sucessful to detect a viral message in leukemic cells of ATL, leading to the hypothesis that the HTLV-I gene expression is not required for the maintenance of T-cell leukemia or lymphoma.

The epidemiological link between HTLV-I and ATLL resulted from two complementary events. The first, an appreciation of Uchiyame and Takatsuki, that ATLL represents a unique clinical entity (9), allowed the geographic localization of the disease to the southern islands of Japan. Epidemiological studies showed that exposure shortly after birth is a major risk factor for subsequent development of HTLV-I associated malignancy (10), while a very small proportion (<5%) of contacts, after a period of twenty or more latent years, develop malignancies, suggesting a multiple step process that may be involved in leukemogenesis, while a viral infection may be a prerequisite, it alone may be insufficient to produce the leukemic phenotype.

These general epidemiologic features of ATLL suggest that systematic reevaluation of appropriate candidate malignancies and viruses may reveal other oncogenic viruses. Careful cataloguing and description of clinical syndromes is essential to derive epidemiological clues that may lead to virus identification. As example: the differentiation of ATLL from mycosis-fungoides is a case in point. While some of the special features of ATLL, as the enhanced expression of interleukin-2 (IL-2) receptor alpha (IL-2Rα) chain (Tac antigen) on the cell surface, was initially ignored while recognition of the subtler clinical aspects of the syndrome allowed for subsequent epidemiologic observations (9). The HTLV-I model for malignancy as a consequence of viral infection suggests that careful molecular reexamination of the role of already recognized viruses in suspect malignancies is in order. In the case of HTLV-I over one million of infected individuals in Japan give rise to about 500 cases of ATLL per year. Hodgkin's disease may provide another case in point. The bimodal age distribution, the age prevalence, anectodal description of geographic clustering and outbreaks of HD suggest possible viral ethiology (11-13).

The increasingly frequent reports of Epstein Bar Virus (EBV) genome detection in some cases of HD suggest that EBV can be important in the pathogenesis of subsets of HD patients (14), as well as in some ENT malignancies (15).

The example of the last five cases of ATLL-like T-cell Lymphoma in Israel, occurring in Iranian immigrants from the area of north eastern Iran (16), raised the possibility of a new population at risk for HTLV-I infection. This is an example of geographic, familial and ethnic clustering of HTLV-I infection that may yield important clues to viral etiology, in view of conflicting reports about clustering in children less than six years of age (17-19) or reports about geographic clustering associated with high socioeconomic status (20-21), while others question those reports (22-23).

In view of the clustering of ATLL in Jewish newcomers from north eastern Iran (16,24) we have designed this study being a systematic survey of HTLV-I serology in newcoming communities to Israel, with an emphasis on middle eastern communities.

The methodology of the research was based on a general survey of Israeli blood donors. Annually about 250.000 units of blood are donated in Israel, big part of them thrqugh the Israel Defence Forces. All data of blood donors is computerized and part of the National Medical Database. This national database is based on the fact that virtually all non-Arab citizens of the country are drafted (25). Conditions in Israel are favorable for epidemiologic research. The country is small, densely populated, with a special ethnic mixture of the Jewish Community and quite society stable population.

Reliable population registers are available and any individual may be traced by his personal, seven digit, identification number, used ago for administrative and research purposes. Data is available from records of hospital admissions and discharges, as well as from the National Compensatory Health Draft Registry, enabling total population studies on Jewish young adults. Numerous epidemiologic studies, done by our group, have used this database (26-34). In addition to conducting an epidemiologic survey, using serologic methods, two additional aimes of this study are to disparate additional disease entities related to HLTV-I and to conduct a molecular survey of HTLV-I.

Approximately five years following the discovery of HTLV-I it was found that the pathology caused by HTLV-I in one setting may not predict other forms of pathology related to the virus. It seems that the role of HTLV-I in ATL is restricted to generating a polyclonal T-lymphocyte proliferation after infection (35).

A specific illustration is afforded by the two major non-overlapping syndromes associated with HTLV-I. While HTLV-I may cause ATLL, another subset of infected individuals that some of them will develop a slow neurologic disease characterized by a gradual development of spastic paresis of the lower extremities with minimal sensory loss. This illness of tropical spastic paresis or HTLV-I-associated myelopathy (HAM), differentially diagnosed from multiple sclerosis, is connected to the virus. The association between the illness – HAM and HTLV-I - was discovered by Gessian et al (36), while screening for neurologic diseases in Martinique. Regardless of underlying mechanism involvement of HTLV-I, in a slow neurologic disease, is quite surpri-

sing. So far, up to the present research, no HAM cases were described in Israel in view of the description of ATLL, an additional aim of our work, as part of the epidemiologic work to screen for possible HTLV-I-associated neurologic disease in Israel.

Serologic survey is backed by western blotting. The demonstration of Seiki and colleagues of the unique coding regions located in the 3' end of the genome provided a first clue as to potential oncologic mechanisms of HTLV-I (37). The 3' ends of the genome of HTLV-I, -II and the leukemogenic bovine leukemia virus (BLV) all contain coding sequences for at least two overlapping genes (38-41). These genes, known as tax and rex, are now known to encode a transcriptional and post-transcriptional regulator of viral expression. The initial discovery of the tax gene was surprising, in that such transacting transcriptional regulators had only been previously identified in DNA viruses such as herpes simplex virus, varicella-zoster virus, and adenovirus.

The HTLV-I tax gene encodes a 40-kilodalton (kDa) protein, and the HTLV-II tax gene encodes a 37-kDa protein (36-41). These nuclear phosphoproteins are highly homologous, and have similar effects on transcription. Tax expression is necessary for transcriptional activation of virus replication and efficient RNA production from the viral long terminal repeat (LTR); however, an additional property noted early was the ability of Tax to trans-activate other viral and cellular promoters, including those involved in regulation of T-cell growth. Examples of such promoters include those for IL-2, IL-2Rα, and the lymphokine, granulocyte-macrophage colony-stimulating factor (GM-CSF) (42-45). In the case of HTLV-I and -II, Tax appears to interact with three 21-base pair (bp) repeats located in the U3 portion of the viral LTR. Within the sequence of the 21-bp repeats are core sequences known to bind cellular transcription factors (46-47). A variety of these proteins have now been identified and partially characterized.

The aim of this research is also to back the serologic and epidemiological work with molecular screening in addition to western blotting, measuring of antibodies to HTLV-I p40tax, with recombinant p40tax as an antigen, since ATL and HTLV-I show the same geographic distribution, and ATL patients are always infected with HTLV-I (48-49,50) it is expected that every new geographic endemic focus will behave similarly, meaning identification of HTLV-I carreirs ATL and HAM population, and viral isolates as part of this study. Moreover, it is important to study the cell surface antigens (CSA) of the isolated infected cells, and viral structure (51).

The genetic structure of HTLV-I is similar to that of the other known retroviruses with three well identified regions: gag region, a pol region and an env region (52-54) with an additional unique pX region (54) that is crucial to the activated host genes in view of those important relations of viral structure and pathogenesis of HTLV-I in particular, and retroviral disease in general, part of our aim was to study viral isolate structure.

FINAL REPORT:

Blood samples were drawn from Israeli blood donors, through the central blood services of Magen David Adom. Additional sources of samples was the collection of blood through the Association of Iranian immigrants to Israel and through the community services of Iranian and Mashadi Jews in Israel: clinics, elderly homes, schools and synagogues in Bney Barak, Holon, Bat-Yam, Herzlia and Tel-Aviv, all towns and communities in the central part of Israel. Additional parts of this survey were samples from patients on long-term hemodialysis, peritoneal dialysis, Adult T-cell Leukemia (ATLL), T-cell malignant disorders, other than Adult T-cell Leukemia, Mycosis fungoides, Sezary's Syndrome Complex, Parapsoriasis, non-Burkitt's Lymphoma patients, young adults with Insulin Dependent Diabetes Mellitus (IDDM) and the collection of blood samples from immigrants from Ethiopia.

During the report period, up to the end of June 1993, we have studied altogether 11230 blood samples including 10122 blood donors (all of them negative for HTLV-I), 480 Iranian Jews of them 212 Mashadi Jews, 181 Ethiopian immigrants, 36 T-cell malignancies, 41 hemodialysis patients, 40 ICPD patients, 32 Mycosis Fungoides/Parapsoriasis patients, 90 non-Burkitt's Lymphoma patients and 208 IDDM patients. Unfortunately, at present we could not collect any data from native Iranians in Mashad or the region, as well as Iranian refugees from Mashad in Europe.

METHODS:

Serologic screening for HTLV-I antibodies in serum or plasma samples was done by means of an enzyme-linked immunosorbent assay-ELISA (Abbott Laboratories). Confirmatory was done by western blotting and/or radioimmunoprecipitation assay - RIPA with Sulphur S^{35}-labeled methionine. HTLV-I-infected HUT 102B lysates were also done (55). Samples, positive in the ELISA, were tested by both confirmatory methods. Antibodies to HTLV-I p40tax were measured by means of an ELISA with recombinant p40 tax as an antigen on solid phase of polystrene beads (Abbott Laboratories Diagnostic Division North Chicago Illinois, USA).

HTLV-I seropositive-infected samples, with a known reactivity against p40tax on RIPA, were used as positive controls (Dr. Rosenblatt's Laboratory Division of Hematology-Oncology Department of Medicine UCLA School of Medicine, Los Angeles California USA) and samples negative for HTLV-I on ELISA and western blot as negative controls. Samples were scored as a positive for p40tax, if the optimal density exceeded 4-5 times the mean negative control value. The polymerase chain reaction - PCR - was used to amplify HTLV-I sequence of DNA from peripheral blood mononuclear cells with primers to a 159bp segment contained with the tax/rex gene as described before (56).

Cell-lines: Lymphocytes were collected from 20ml of heparinized peripheral blood by a Ficoll-Hypaque density centrifugation and resuspended in RPMI-1640 medium supplemented with 15% fetal bovine serum, interleukin-2 (IL-2) at a concentration of 50u/ml, 0.5% PHA and 1% penicillin/streptomycin. Cells were incubated at $37^{o}C$ in the presence of 5% CO^{2} and were maintained for several weeks. The cells were cryopreserved at various intervals for DNA extraction.

DNA extraction and PCR amplification: DNA was extracted from lymphocytes with phenol/chloroform. Two regions in the HTLV-I genome were amplified: LTR and env gene. LTR amplification was performed with the primers R11/14 which flank a 741 base sequence from nucleotide 61 to 802 (nucleotides are numbered according to the sequence of Seiki et al (37). The env gene was amplified by two pairs of primers: R15/17A which flanks a 1163 base sequence from nucleotide 5201 to 6364 and R19/18 primers which flank 791 base sequence from nucleotide 5942 to 6733. The patient's (HE) DNA was PCR amplified for 30 cycles in a reaction containing 100 µl of 2nM MgCl, 200 ng from each primer, 0.2 mM from each dNTPs and 2.5 u of taq polymerase (ESB, Cleveland, OH). A list of primers used for PCR amplification is presented in table 1.

DNA sequencing: PCR products were recovered from the PCR reaction with DS primer remover (Advanced Genetic Technologies Corp. Gaithersburg, MD) and ethanol precipitation. Nucleotide sequence analysis was performed by the bTaq Dye Deoxy Terminator Cycle Sequencing kit (Applied Biosystems) using the 373A DNA sequencer (Applied Biosystems)

at the Biological Services of the Weizmann Institute Rehovot Israel. Sequence analysis was performed at least twice for each primer (Table 1).

Sequence comparison: HE sequence was compared to the Japanese (ATK), African (Zair, EL) and Papua New Guinea (PNG-1) sequences by geg program with the accession numbers: J02029 (ATK), S74562 (Zairian LTR), M85207 (PNG-1) and M69044 (EL).

Primer	From nt.	To nt.	Used for	Seq.	Remarks
R11	61	77	amp. & seq.	5' TAGAGCCTCCCAGTGAA	
R12	494	470	seq.	5' CCTAGACGGCGGACGCAG	comp.
R14	802	786	amp. & seq.	5' CTCGTATCCCGGACGAG	comp.
R15	5201	5218	amp. & seq.	5' CATGGGTAAGTTTCTCGC	
R16	5660	5645	seq.	5' ATGGAGATTAATATTG	comp.
R17	5641	5658	seq.	5' GCCTCAATATTAATCTCC	comp.
R19	5942	5959	amp. & seq.	5' TCCATCCTCTTCTTCTAC	
R17A	6364	6347	amp.	5' TCCCAGAACAGGAGATCA	comp.
R18	6733	6716	amp. & seq.	5' GGGAGAGGTAATTATTG	

able 1: list of primers for PCR amplification and sequencing.

RESULTS:

Out of 11230 blood samples tested for HTLV-I we have found 26 positive for HTLV-I in ELISA and western blot and 12 positive in anti p40tax ELISA. The positive results are detailed in the following tables.

TABLE 2: Prevalence of HTLV-I infection

Patients	HTLV-I & western blot	
	# tested	# Positive (%)
Iranian Jews	480	5.41% (26)*
Mashadi Jews	212	12.3% (26)
Ethiopian Jews	181	0 %
T-cell malignancy	136	0%
Haemodialysis	41	0%
CIPD	40	0%
Mycosis Fungoisis & Parapsoriasis	32	0%
Non-Burkitt's Lymphoma	90	0%
IDDM	208	0%

*All positive patients were of Mashadi origin

TABLE 3: Anti p40tax ELISA results

Patients	Anti p40tax ELISA	
	# tested	# Positive (%)
Mashadi Jews	190	19 (10%)
Other Iranian Jews	92	0%
Ethiopian Jews	65	0%
Haemodialysis	20	0
CIPD	40	0
Mycosis Fungoisis & Parapsoriasis	30	0%
Non-Burkitt's Lymphoma	22	0%

The extensive social and ethnic ties between Jews of Mashadi origin allowed selective sampling of this population within Israel. On repeated assys, 26 of 212 (12.3%) Mashadi serum samples were positive by ELISA for antibodies to HTLV-I and were confirmed by western blotting. In contrast, none of the control samples was positive by ELISA (Tables).

In an effort to survey the oldest members of the Mashadi population, we tested 32 unrelated elderly Mashadi women living in an old people's home in Tel-Aviv, 14 (44%) were seropositive, a rate three times higher than that in the general Mashadi population. At our first sample collection, 12 (52%) of 23 long-standing residents of the home were seropositive, 2 of 9 new residents surveyed a year later were seropositive. Of 26 Mashadi women, older than 60 years, who lived elsewhere in Israel only 3 (12%) were seropositive.

To determine whether serologocal assays had detected all infected subjects, we carried out PRC amplification of HTLV-I DNA from 29 of the residents of the old people's home. 19 were positive for HTLV-I sequences: 14 of these were seropositive, 4 were seronegative, and 1 was seronegative by standard ELISA but reactive in the anti-p40tax ELISA. A second PCR on repeat samples from 10 of these women showed concordant results in 6 of 6 PCR-positive and 2 of 3 PCR-negative cases; 1 woman originally positive on PCR was negative on the second sample, and 1 woman originally PCR-negative was faintly positive on her second sample and had antibodies to p40tax by ELISA. This unusual clustering of HTLV-I infection within the old people's home suggested acquisition of infection within the institution.

In addition to antibodies to virion components, we assayed for antibodies to the non-structural p40tax protein. Of 190 Mashadi samples tested 19 had absorbance levels 4-5 or more times those of the negative control and were judged positive (Tables). 92 samples were negative by both assays, 8 samples were seropositive for both anti-HTLV-I and anti-p40tax; 13 samples were positive for anti-HTLV-I and negative for anti-p40tax; and 4 were positive for anti-p40tax but repeatedly negative for anti-HTLV-I by ELISA and western blot. 3 of the latter group of samples were negative on PCR and the other, from a resident of the old people's home, was positive. All 4 were negative by western blotting and by RIPA. Therefore, the reason for the high titres in three samples in the anti-p40tax ELISA is unclear.

Direct comparison of anti-p40tax ELISA results and those of the RIPA was done for 28 subjects. 8 samples were positive for anti-p40tax by both RIPA and ELISA; 1 with traces of antibody by RIPA was negative by ELISA; the 3 samples mentioned previously were negative by RIPA but repeatedly positive by ELISA and 16 were negative by both tests. The usefulness of the anti-p40tax ELISA in detecting true additional HTLV-I-infected seronegative individuals remains unclear, since seronegative infection was corroborated by other means of detection in only 1 of the 4 questionable cases.

The finding of a higher rate of seropositivity among elderly Mashadi women, than in the general Mashadi population, suggested a potential for gradual development of seropositivity with age, as has been reported by others. Because of the possibility that serological screening may not detect all positive subjects we carried out HTLV-I-specific PCR on DN from 68 randomly selected Mashadi blood samples. 5 of 7 known seropositive samples were positive in the PCR assay, and 61 sero-negative samples were all negative by PCR. Hence, PCR did not increase sensitivity of detection within this sample above that was achieved with serological assays alone. All PCR-positive samples contained HTLV-I and not HTLV-II, as shown by discriminatory PCR.

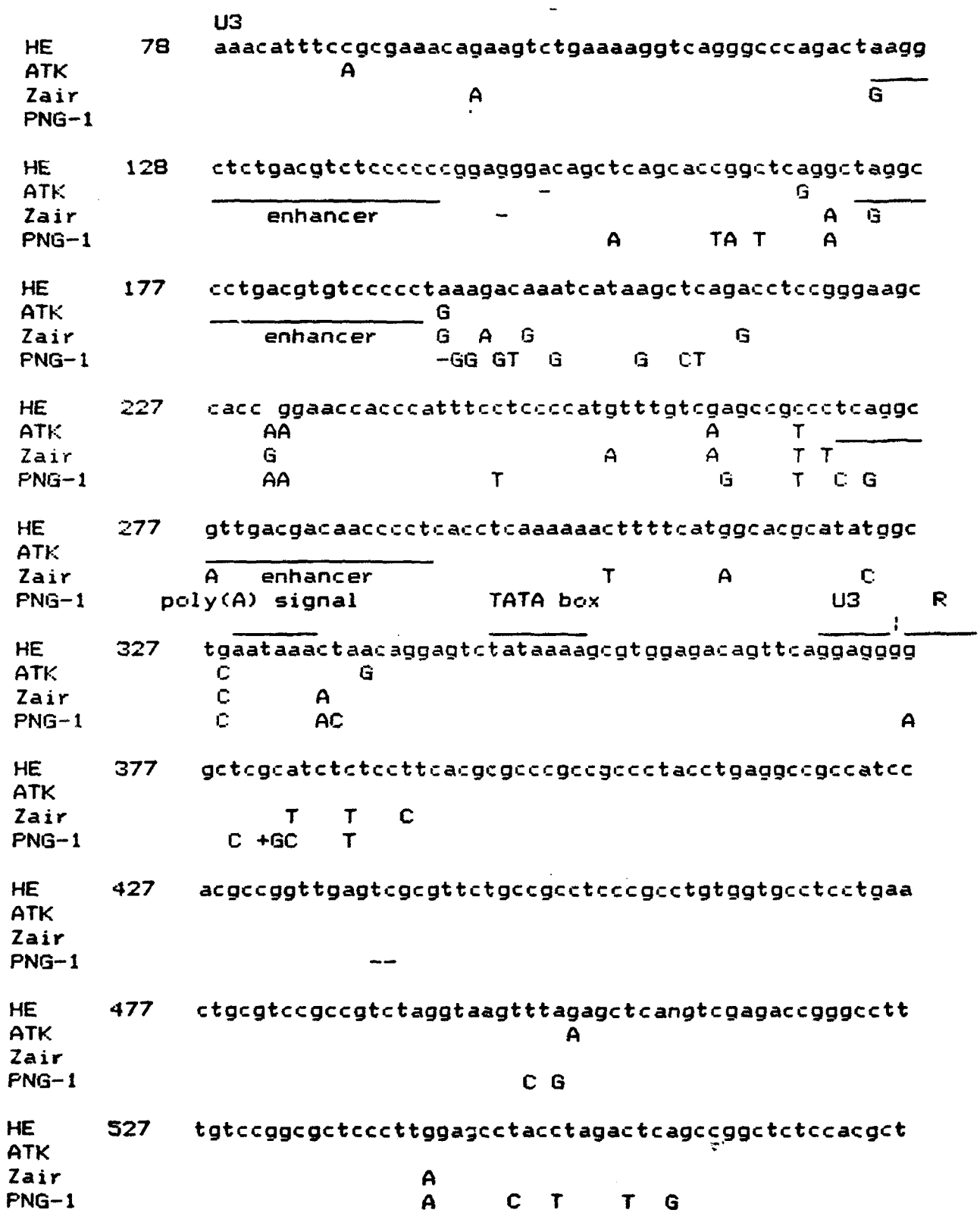
We have characterized the Iranian HTLV-I isolate using a combination of Southern blotting, PCR and sequencing. We have developed a primary T-cell line from a Mashadi HTLV-I carrier containing integrated HTLV-I provirus.

Comparison of HTLV-I(HE) LTR to other isolates: PCR amplification of HTLV-I LTR produced a PCR product of 741bp of which 698 bases were sequenced. 7 nucleotides, which are not verified yet, are designated as N. HTLV-I (HE) LTR sequence was compared to three other isolates. The Japanese (ATK) sequence showed nucleotide homology of 97.8% with 15 nucleotide differences (37). The Zairian sequence showed nucleotide homology of 95% with 35 nucleotide differences and the Papua New Guinea isolate showed 91% homology with 56 nucleotides differences, in 629 nucleotides examined (56,57,58).

Comparison of the sequence changes in the LTR region to the Japanese sequence showed that the differences are clustered mostly in the U3 (66.7%), 6.7% were in the R and 26.7% were in the U5 regions. There are no changes at the three 20bp enhancer elements at the U3 region and there is 98.7% homology for the Rex responsive element located between bases 313 and 627 (Fig. 1,2).

Comparison of the env gene amplified from HTLV-I (HE) genome to the Japanese and African sequences. The PCR product was 1532 bp in length of which 1496 bases were sequenced. The sequence between nucleotides 5625-5685 is not verified yet (Fig. 3).

Comparison to the Japanese 'ATK sequence showed nucleotide homology of 97.7% with 9 nucleotide differences, while the African (EL) sequence showed nucleotide homology of 98.4% with 6 nucleotide differences. There is a problem with base T position 5400. It seems that it creates a stop codon while in the other two sequences it does not exist. This finding needs further examination, but may be due to a defective HE provirus or PCR cloning artifact. HE env2 sequence was compared to the Japanese (ATK) sequence (37). 98.7% homology at the nucleotide level with 12 nucleotide differences were found. Comparison to the African (EL) sequence showed nucleotide homology of 99.5% with 5 nucleotide differences (58,59). In contrast, comparison of nucleotide sequences 5424-5839 within env between the Iranian isolate and MEL5, a Melanesian isolate from the Solomon Islands shows 11 nucleotide changes, as compared to none between the Iranian and Japanese (ATK) isolates (V. Nerurkar, pers. communication).


U3
HE 78 aaacatttccgcgaaacagaagtctgaaaaggtcagggcccagactaagg
ATK A
Zair A G
PNG-1
HE 128 ctctgacgtctcccccccggagggacagctcagcaccggctcaggctaggc
ATK G
Zair enhancer A G
PNG-1 A TA T A
HE 177 cctgacgtgtcccccctaaagacaaatcataagctcagacctccgggaagc
ATK G
Zair enhancer G A G G
PNG-1 -GG GT G G CT
HE 227 cacc ggaaccacccatttcctcccccatgtttgtcgagccgccctcaggc
ATK AA A T
Zair G A A T T
PNG-1 AA T G T C G
HE 277 gttgacgacaaccccctcacctcaaaaaactttttcatggcacgcatatggc
ATK
Zair A enhancer T A C
PNG-1 poly(A) signal TATA box U3 R
HE 327 tgaataaactaacaggagtctataaaagcgtggagacagttcaggagggg
ATK C G
Zair C A
PNG-1 C AC A
HE 377 gctcgcatctctccttcacgcgcccgccgccctacctgaggccgccatcc
ATK
Zair T T C
PNG-1 C +GC T
HE 427 acgccggttgagtcgcgttctgccgcctcccgcctgtggtgcctcctgaa
ATK
Zair
PNG-1 --
HE 477 ctgcgtccgccgtctaggtaagtttagagctcangtcgagaccgggcctt
ATK A
Zair
PNG-1 C G
HE 527 tgtccggcgctcccttggagcctacctagactcagccggctctccacgct
ATK
Zair A
PNG-1 A C T T G

```
                                                R     U5
                                               ____!____          poly(A))
HE      577   ttgcctgaccctgcttgctcaactctgcgtctttgtttcgttttctnttc
ATK                                      A
Zair                        T       C    A
PNG-1                   -                CA

HE      627   tgcgccgctacagatcgaaagttccacccctttcccnnncattcncgact
ATK                 T
Zair                T
PNG-1               T   C               G                      T

HE      677   gactgccggcttggcccacggccaagtaccggcgactccgttggctcgga
ATK
Zair                                             T     TTAC
PNG-1                                        --      AC   T AC

HE      727   gccagcgacagcccattctatagcactctccaggagagaaanttagtaca
ATK                           C              -
Zair                        T                                 C
PNG-1                                 C T                   A   A

HE      776   c
```

Fig. 1: Nucleotide sequence of HTLV-I(HE) LTR. Nucleotide differences from the Japanese (ATK), Zairian (Zair) and Papua New Guinea (PNG-1) sequences are shown underline. Nucleotides are numbered according to ATK sequence

```
                  SU
HE    5219    cactttgattttattcttccagttctgccccntcatcctcggtgattaca
ATK                                                C
EL                  A

HE    5269    gccccagctgctgtactctcacaattggagtctcctcataccactctaaa
ATK
EL

HE    5319    ccctgcaatcctgcccagccagtttgttcgtggaccctcgacctgctggc
ATK
EL

HE    5369    cctttcagcagatnaggccctacagccccctngccctaatctagtaagt
ATK                                        -       C
EL                                         -             G

HE    5418    tactctagctaccatgccacctattccctatatctattccctcattggat
ATK                C                                          C
EL                        T

HE    5468    taaaaagccaaaccgaaatggcggaggctattattcagcctcttattcag
ATK                G
EL

HE    5518    acccttgttccttaaagtgcccatacctggggtgncaatcatggacctgc
ATK
EL

HE    5568    ccntacanaggagncgnctncaggccntactggnagtttcannaagatgt
ATK                T                    C                   C
EL                 T                    C

HE    5618    naatttt
```

Fig. 2: Comparison of HE env1 sequence to the Japanese (ATK) and African (EL) sequences.

```
HE    5686    cccttctagtcgangntccagganatgaccccatctggttccttaatacc
ATK
EL

HE    5736    gancccagncaactgnctcccaccgnccctcctctactcccccactctaa
ATK
EL

HE    5786    nctagancacatcctcgagccctctataccatggaaatcaaaactcctga
ATK
EL

HE    5836    cccttgnccagttaaccctacaaagcactaattatacttgnattgnctgt
ATK
EL

HE    5886    atcgatcgtgncagnctatccacttggnacgtcctatannntcccaacgt
ATK                                C
EL

HE    5936    ctctgntccatccncttcttctacccccccncctttacccatcgttagcgc
ATK
EL

HE    5986    ttccagcccccccacctgacgttaccatttaactggacccactgctttgac
ATK
EL

HE    6036    ccccagattcaagctatagtctcctcccccctgtcataactccctcatcct
ATK
EL

HE    6086    gccccccttttccttgtcacctgttcccaccctgggatcccgctcccgcc
ATK                                                  A
EL          SU    TM                                 A
            ____:____
HE    6136    gagcggtaccggtggcggnctggcttgtctccgccctggncatgggagcc
ATK
EL

HE    6186    ggagtggctggcgggattaccggctccatgtccctcgcctcaggaaagag
ATK
EL

HE    6236    nctcctacatgaggtggacaaagatatttcccaattaactcaagcaatag
ATK                                                  G
EL

HE    6286    tcaaaaaccacaaaaatctnctcaaaattgcgcagtatgctgcccagaac
ATK
EL                  .

HE    6336    agacganggcnngatctcctgttctgggagcaaggaggattatgnaaagc
ATK                     C
EL                      C
```

```
HE    6386    attacaagaacagtgctgttttctgaatattactaattccnatgtctcna
ATK                         C       C           C              -
EL

HE    6435    atactacaagaaagacccccccttgagaatcgagtcctgactggctgggg
ATK
EL

HE    6485    ccttaactgggaccttggcctctcacagtgggctcgagaggccttacaaa
ATK
EL

HE    6535    ctggaatcacccttgtcgcgctactccttcttgtcatccttgcaggacca
ATK                                                 T
EL                                                  T

HE    6585    tgcatcctccgtcagctacggcacctcccctcgcgcgtcagatacccccca
ATK                               A
EL                                A

HE    6635    ttactctcttataaaccctgagtcatnccctgtaaaccaagcacacaatt
ATK                           A                             G
EL                                    -

HE    6684    attgcaaccacatcgcctccagcctcccctgc
```

Fig. 3: Comparison of HE env2 sequence to the Japanese (ATK) and African (EL) sequences.

DISCUSSION:

We have identified a high risk of HTLV-I infection in Iranian Jews originating from the city of Mashad in Khurusan, northeastern Iran. This group seems to have an overall infection rate of about 12%, though the rate was much higher among residents of a Mashadi home for old people. The reason for this clustering is unclear, although the findings suggest acquisition of infection within the institution. The overall level of infection among other Iranian Jews seems to be substantially lower than that among Mashadis. The explanation may be geographic, and Mashad may be within a previously unrecognised endemic region. Since we did not test non-Jewish Mashadis, this possibility cannot be excluded. In the present situation we will not be able to test this population unless USAMRD, or any other federal agency, may help us with a proper connection with the Mashad University or any other scientific group in Iran.

The unique history of the Mashadi community may help to explain their high rate of HTLV-I infection. In 1839, the Jewish community in Mashad was subjected to a series of violent attacks and forced to convert to the Islam, though the majority of the community continued to practise Judaism covertly (62). To safeguard the community secret necessitated a very high rate of intermarriage among community members. Members of this community continued to marry close relatives for over the next 150 years. Markers of consanguinity are high among Mashadi Jews - for example, over 20% have a deficiency of glucose-6-phosphate dehydrogenase (63). The chance introduction of HTLV-I infection into the community could have resulted in rapid transmission of the virus by sexual means and by breastfeeding.

It was recently shown by Krivine (64) that there is an retrovirus replication in the first weeks of life. In a prospective longitudinal study of 50 infants, born to HIV-seropositive women, blood samples were obtained at birth at 4-9 weeks, and 5-9 month of age and were tested for HIV RNA by the polymerase chain reaction (PCR), viral culture and p24 antigen measurement. 16 were diagnosed as HIV infected by the age of 4-9 weeks by PCR and culture, while only 10% of the newly born - 5 were detected as HIV-positive at birth. No changes

in HIV infection were detected on both ages of testing for HIV. We started a similar study in HTLV-I-seropostive women, assuming that perinatal HTLV-I infection could also be demonstrated by PCR or culture , after the first two months of life. Such a work may also give some clues about the ways of transmission of HTLV-I and possibility of HTLV-I-like HIV during the first weeks of life, unfortunately on writing this final report we still do not have HTLV-I neonatal data from Israel.

An estimated 5000-6000 Mashadis now live in Israel. This is the first Israeli ethnic group identified as having a high rate of HTLV-I infection. Members of the Mashadi Jewish community also migrated to the USA and parts of Europe. We estimate that similar prevalence of HTLV-I infection could be found in other Jewish Mashadi communities. We intend to continue this work during the next year, and formed already initial conncetion with the Mashadi community in Milano, Northern Italy. Unfortunately we cannot do any work at present with native Iranian residing in Mashad.

In addition, it is estimated that Mashadi and Iranian Jews have moved to the East, to the regions of Uzbekistan, Afganistan, Kazahstan, Armenia and Pakistan (supplement map). Initial study of samples of those newly coming immigrants from Eastern Soviet republics (parts of former USSR) were negative but the data is still to small. We estimate that at least 20.000 immigrants have arrived to israel from those areas. There was also a settlement of Iranian Jews in China, in the area of Kaifen. But at present we have no possibility to explore this community, that partly assimilated in the general Chinese population.

In this study we use several methods to detect HTLV-I infection. Our rate of anti-p40tax seropositivity in carriers was somewhat lower than that found by others among healthy HTLV-I carriers (63-66), independent evidence for infection using PCR was obtained only in 1 out of 4 samples, thus detection of anti-p40tax antibodies did not appreciably add to the estimate of the rate of infection, and there is no point of its further use in our study. Our findings on the use of PCR suggest that in a high risk population such as the Mashadi population in the old people's home we have studied, or in families with HTLV-I

carriers, PCR would increase the number of detected infected individuals above those that are detected by serological survey.

The results in general population survey, in quite big sample, suggests that there is no point to continue such screening in high risk populations only. The usefulness of PCR as a screening assay in approriate setting requires further study.

The limited number of Ethiopian Jews studied does not confirm previously published results (67) about HTLV-I infection in the Falashi community and further work is needed in this group too. We have not summarized, yet, the data of Mycosis Fungoides and Parapsoriasis patients. Further work is also needed with the study of Mashadi isolates and their sequence as compared to Japanese and other isolates. The successful identification of HTLV-II by vitro of limited nucleic acid homology and antigenic similarity to HTLV-I suggests that variants and new retroviruses can be identified. We have published our initial result in a paper attached to this report (68). We have determined the sequence of two regions in HTLV-I isolated in Israel from an HAM patient belonging to this community: the LTR region and the env gene.

Several studies have indicated a high degree of homology among HTLV-I isolates (>96%) and demonstrated that differences between variants are primarily associated with their geographical origin (69-70). We compared HTLV-I in an Iranian immigrant with HAM to the Japanese, African and Papua New Guinea isolates. A high level of homology of about 98% was observed to the Japanese isolate, while the African and Papua New Guinea isolates were more divergent with sequence homology of 95% and 91% in the LTR respectively.

We have summarized, recently, our sequence data appearing in the appendix of this report (71-72).

Comparison of HTLV-I env gene to the Japanese and African isolates showed homology at the nucleotide level of about 98-99%. From these results we can conclude that the env gene is more conserved than the LTR region.

With the exeptions of some nucleotides which are not verified yet, comparison of the LTR region showed the highest homology between the Iranian and Japanese isolate. As for the env gene, the Iranian isolate was most similar to the Japanese isolates. Therefore, these results suggest that the Iranian isolate is phylogenetically most similar to the prototypic Japanese/African isolates, and divergent from the Melanesian variants of HTLV-I.

There are two theories about HTLV-I origin and the way it was spread to the world. Gallo suggests that it originated in Africa and spread to the new world and Japan, while Saskena et al. suggest that HTLV-I originated in the Indo-Malay region (56,67-68). Based on the comparison of the LTR sequences, our results support the theory of HTLV-I may have originated in Africa, since we found a higher degree of homology between the HE sequence and the Zairian isolate, than with the sequence of Papua New Guinea isolate. It is likely that HTLV-I may have reached Iran from Africa by overland trade routes traversing through Mashad. These routes may have extended to the Far East (Japan) rather than the more commonly held notion of seaborne extension to Southern Japan, thus linking the African/Iranian and Japanese isolates. Comparative sequence analysis once again confirms the striking homology between distant HTLV-I isolates compared to HIV-I.

Over a decade following initial identification of the role of the retrovirus in human disease, HTLV-I remains as the only example for human retroviral leukemogenesis. Several viruses can be derived from the HTLV model. First, leukemia may be the rare consequence of infection with a relatively common virus. Second, a long latency period may be involved following acquisition of infection, and the leukemogenic process may involve multiple steps beyond initial infection. Third, the mechanism by which the virus may predispose to development of leukemia may be different from that seen with other retroviruses, and may involve transacting viral genes, either regulators of DNA transcription or regulators of viral mRNA expression. Finally, the role of HTLV-I as an etiologic agent in both HAM and ATLL suggests that viruses, not thought to have oncogenic properties, may on occasion predispose to malignancy. The overall lesson from the HTLV experience is that when suitable or suggestive epidemiologic

factors are found, such as ethnic, geographic and/or familial clustering in the absence of a known inheritance pattern, a search for underlying viruses should be initiated. The search would involve removal of malignant tissue, when possible, for culture in vitro and a search using available molecular and serologic probes.

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CONCLUSIONS

During the two years of the project, summarized in this final report, we have studied more than 11.000 blood samples for HTLV-I serology and molecular detection and characterization of the virus.

We have been successful in identifying a new high risk population for HTLV-I in the Middle East, a population of immigrant Jews from Iran, from the region of Khurusan (North East Iran - NEI) and mainly from the town of Mashad. It seems that HTLV-I infection is prevalent among this special community, like some other areas of the world, like southern Japan and Pacific Islands, several Caribbean countries and central Africa. The prevalence of HTLV-I infection in native Iranians and large population of Iranians and Mashadies residing in North America and Europe has not been studied yet.

Because persons infected with HTLV-I may be screened medically for HTLV-I associated diseases and because changes in breast feeding, sexual behavior, and blood donation can interrupt the transmission of the virus, studies of this kind have a major importance in preventive medicine.

This special population, with first description and virus isolates of Adult T cell Leukemia (ATTL) and HTLV-I Associated Myelopathy (HAM) gives a special and new insight on HTLV-I epidemiology, with special interest focusing on a concentration of HTLV-I positive patients, in a very senior population residing in a geriatric center.

Various serological and molecular screening methods, including enzyme-linked immunosorbent assay (ELISA) for anti-HTLV-I, ELISA for an antibody for recombinant HTLV-I p40tax protein, and molecular detection of infection by a polymerase chain reaction (PCR) amplification of HTLV-I proviral DNA, from peripheral mononuclear cells DNA, were used.

By HTLV-I ELISA the overall rate of infection was 12.2% among immigrants from Khurusan (northeastern Iran), non HTLV-I carriers were detected in a general survey of the population and other high risk groups, including other Iranian and Ethiopian Jews, as well as some clinical conditions such as ATLL, other T-cell malignancies and haemodialysis patients. We have found an unexplained clustering of HTLV-I infection in a cohort of 32 elderly women of similar geographic origin (Mashad) in a home of senior citizens, 14 were seropositive in ELISA and 19 of 28 were positive by PCR. These findings and this newly identified high risk population suggests that, in addition to ELISA, other screening techniques may be required to detect all carriers in high risk populations.

We have done detailed sequencing work on an HAM Iranian isolate of HTLV-I virus.

In this study we determined the sequence of two regions in HTLV-I isolated in Israel from an HAM patient belonging to this community: the LTR region and the env gene. Several studies have indicated a high degree of homology among HTLV-I isolates (>96%) and demonstrated that differences between variants are primarily associated with their geographical origin. We compared HTLV-I in an Iranian immigrant with

HAM to the Japanese, African and Papua New Guinea isolates. A high level of homology of about 98% was observed to the Japanese isolate, while the African and Papua New Guinea isolates were more divergent with sequence homology of 95% and 91% in the LTR respectively.

Comparison of HTLV-I env gene to the Japanese and African isolates showed homology at the nucleotide level of about 98-99%. From these results we can conclude that the env gene is more conserved than the LTR region.

With the exeptions of some nucleotides which are not verified yet, comparison of hte LTR region showed the highest homology between the Iranian and Japanese isolate. As for the env gene, the Iranian isolate was most similar ot the Japanese isolates. Therefore, these results suggest that the Iranian isolate is phylogenetically most similar to the prototypic Japanese/African isolates, and divergent from the Melanesian variants of HTLV-I.

There are two theories about HTLV-I origin and the way it was spread to the world. Gallo suggests that it originated in Africa and spread to the new world and Japan, while Saskena et al. suggest that HTLV-I originated in the Indo-Malay region. Based on the comparison of the LTR sequences, our results support the theory of HTLV-I may have originated in Africa, since we found a higher degree of homology between the HE sequence and the Zairian isolate, than with the sequence of Papua new Guinea isolate. It is likely that HTLV-I may have reached Iran from Africa by overland trade routes traversing through Mashad. These routes may have extended to the Far East (Japan) rather than the more commonly held notion of seaborne extension to Southern Japan, thus linking the African/Iranian and Japanese isolates. Comparative sequence analysis once again confirms the striking homology between distant HTLV-I isolates compared to HIV-I.

HTLV-I has been recently associated with some new additional disease, like HTLV-I associated arthropathy, HTLV-I polymyositis, HTLV-I uneitis, and pediatric HTLV-I infectious dermatitis. Unfortunately, we have not been able at this stage to detect any of those, and our studies in IDDM newly diagnosed patients, non-Burkitt's Lymphoma patients, and Psoriasis/Parapsoriasis patients were all negative.

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1. Benjamin Kornbrot, M.D.
2. Elisabeth Kaminsky, M.Sc.
3. Yael Kilim, M.Sc.
4. Merav Crook

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HTLV-I INDUCED T-CELL LYMPHOMA IN ISRAELI PATIENTS OF IRANIAN ORIGIN

J. Rosenblatt, D. Meites, Y. Sidi, Y.L. Danon

Rogoff-Wellcome Medical Research Institute, Dept. of Medicine and Div. of Pediatric Immunology, Edith Wolfson Hospital and Beilinson Medical Center Tel-Aviv University Sackler School of Medicine, Petah-Tikva 49100 ISRAEL.

T cell lymphoma-leukemia (ATL) is one of the several clinical entities linked to human T cell lymphotropic virus (HTLV-I). Few geographic endemic concentrations of HTLV-I infection were already described: The Ryukyu Islands in Southern Japan, Central Africa and the Caribbean Islands. This is the first description of endemic HTLV focus in the Middle East. The prevalence and clinical presentations of ATL in Israel were studied. We have diagnosed four Israeli Jewish ATL patients and one HAM (HTLV-I - Associated Myelopathy) in a nationwide survey performed in 1986-1990. In three of the patients evidence for HTLV-I infection was obtained. All those patients immigrated to Israel from the same region in Central Iran. The nationwide survey and the clinical course of this new group of patients will be presented.

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MOLECULAR CHARACTERIZATION OF IRANIAN HTLV-I ISOLATES

Y. Kilim[1], J.D. Rosenblatt[2], D. Meytes[3], D. Stephens[2], H. Lee[4], Y. Danon[1]
Children's Medical Center of Israel, Petach, Tiqva, Israel[1], UCLA School of Medicine, Los Angeles, CA[2], Edith Wolfson Hospital, Holon, Israel[3], Abbott Laboratories, N. Chicago, IL[4], USA

We recently reported a high rate of HTLV-I seropositivity among immigrants to Israel from Mashad in Northeastern Iran (Lancet 336:1533-1535, 1990). We have now characterized the Iranian HTLV-I isolate using a combination of Southern blotting, polymerase chain reaction (PCR) and sequencing. DNA from 10 HTLV-I seropositive Mashadi carriers was isolated from peripheral blood mononuclear cells and amplified by PCR using primers to the tax/rex region. All 10 samples were found to be HTLV-I and not HTLV-II using discriminatory PCR. An immortalized T-cell line was derived from a Mashadi HTLV-I carrier. The cell line contained integrated HTLV-I provirus. Southern blotting and restriction mapping of the Iranian isolate demonstrated marked similarity to published Japanese isolates using 10 different restriction enzymes. Amplification and preliminary sequencing of a 900 bp segment of env from two Mashadi isolates disclosed approximately 95% nucleic acid sequence homology with the published sequence of Japanese isolates, and >98% homology between two Mashadi isolates. These data indicate substantial conservation of sequence between Iranian and Japanese HTLV-I isolates. Further characterization of Iranian HTLV-I isolates is underway. (Supported by the Rashi Foundation, Doron Foundation, ICRF, and a grant from the USAMRDC).

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EPIDEMIOLOGIC AND MOLECULAR CHARACTERIZATION OF HTLV-I INFECTION IN ISRAEL

Yehuda L. Danon, Yael Kilim and J. Rosenblatt, Kipper Institute of Child Immunology, Children's Medical Ctr. of Israel, Sackler School of Medicine, Tel-Aviv Univ. Kaplan Str. #14-16, Petah-Tikva 49100 Israel.

Human T-cell leukemia viruses type I (HTLV-I) and type II (HTLV-II) are the two human retroviruses directly implicated in pathogenesis of human leukemia. HTLV-I has been linked to adult T-cell leukemia/ lymphoma (ATLL), and HTLV-II to rare cases of chronic T-cell leukemia. Epidemiologic and molecular studies of both viruses have identified several themes underlying the leukemogenic process. Leukemia is a rare consequence of infection, and both viral infection and secondary transforming events appear to be involved. Due to the absence of a virally encoded oncogene, or preferred viral integration sites, novel mechanisms of leukemogenesis must be invoked. We recently reported a high rate of HTLV-I seropositivity among immigrants to Israel from Mashad in Northeastern Iran (Lancet 336:1533-1535,1990). We have now characterized the Iranian HTLV-I isolate using a combination of Southern blotting, polymerase chain reaction (PCR) and sequencing. DNA from 10 HTLV-I seropositive Mashadi carriers was isolated from peripheral blood mononuclear cells and amplified by PCR using primers to the tax-rex region. All 10 samples were found to be HTLV-I and not HTLV-II using discriminatory PCR. An immortalized T-cell line was derived from a Mashadi HTLV-I carrier. The cell line contained integrated HTLV-I provirus. Southern blotting and restriction mapping of the Iranian isolate demonstrated marked similarity to published Japanese isolates using 10 differrent restriction enzymes. Amplification and preliminary sequencing of a 900 bp segment of env from two Mashadi isolates disclosed approximately 95% nucleic acid sequence homology with the published sequence of Japanese isolates, and >98% homology between two Mashadi isolates. These data indicate substantial conservation of sequence between Iranian and Japanese HTLV-I isolates. Further characterization of Iranian HTLV-I isolates is underway. A potential role for the HTLV-I/II transregulatory proteins Tax and Rex in leukemogenesis is discussed. Insights derived from the study of HTLV-I/II can be applied to the search for other oncogenic viruses.

(Supported by the Rashi Foundation, Doron Foundation, ICRF, and a grant from the USAMRDC).

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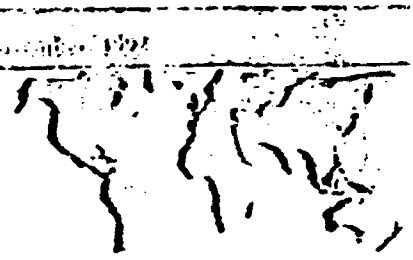

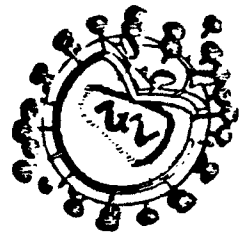

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(Supported by the USAMRDC and Doron Foundation)

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Viruses and Virus-Like Agents in Disease

Molecular characterization of HTLV-I infection in Israel

Yehuda L. Danon, Yael Kilim and Joseph Rosenblatt
Kipper Inst. of Child Immunology, Children's Med. Ctr. of Israel, Sackler School of Med., Tel-Aviv Univ. Israel

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Supported in part by USAMRDC Grant # DAMD17-91-C-1001

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MOLECULAR CHARACTERISTICS OF HTLV-I INFECTION IN NEWLY CHARACTERISED HIGH RISK GROUP OF CARRIERS IN THE MIDDLE EAST.

HTLV-I has been linked to adult T-cell leukemia/lymphoma (ATLL), and HTLV-II to rare cases of chronic T-cell leukemia. We recently reported a high rate of HTLV-I seropositivity among immigrants to Israel from Mashad in Northeastern Iran. We have now characterized the Iranian HTLV-I isolate using a combination of Southern blotting, polymerase chain reaction (PCR) and sequencing. DNA from 10 HTLV-I seropositive Mashadi carriers was isolated from peripheral blood mononuclear cells and amplified by PCR using primers to the tax-rex region. All 10 samples were found to be HTLV-I and not HTLV-II using discriminatory PCR. The cell line contained integrated HTLV-I provirus. Southern blotting and restriction mapping of the Iranian isolate demonstrated marked similarity to published Japanese isolates using 10 different restriction enzymes. Amplification and preliminary sequencing of a 900 bp segment of env from two Mashadi isolates disclosed approximately 95% nucleic acid sequence homology with the published sequence of Japanese isolates, and >98% homology between two Mashadi isolates. These data indicate substantial conservation of sequence between Iranian and Japanese HTLV-I isolates. Insights derived from the study of HTLV-I/II can be applied to the search for other retroviruses and oncogenic viruses.

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EPIDEMIOLOGIC AND MOLECULAR CHARACTERIZATION OF HTLV-I INFECTION IN ISRAEL

Yehuda L. Danon, Yael Kilim and Joseph Rosenblatt, Kipper Institute of Child Immunology, Children's Medical Center of Israel, Sackler School of Medicine, Tel-Aviv University Israel

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HTLV-I VIRUS IN INSULIN-DEPENDENT DIABETES MELLITUS

Y. Kilim, M.Sc.[1], M. Karp, M.D.[2] and Y.L. Danon, M.D.[1]
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Human T-cell Leukemia Virus-I has been linked to adult T cell leukemia/lymphoma (ATLL) and HTLV-II to some cases of chronic T cell leukemia. We have recently reported a high rate of HTLV-I seropositive among immigrants to Israel from northeastern Iran, and especially the town of Mashad.

To determine the frequency of antibodies to HTLV-I virus in Insulin-Dependent Diabetes Mellitus (IDDM) patients, sera from 56 newly onset IDDM patients were tested by an enzyme immunoassay. According to our method the reactivity of antibodies detected by enzyme immunoassay against HTLV-I encoded antigens was determined by an assay which employs recombinant HTLV-I antigens. No antibodies to HTLV-I were detected in all 56 patients studied. Proliferative response to various species of insulin was performed in 26 of those patients, 23 out of 26 showed a positive response. Sera from 56 newly onset IDDM patients were screened for ICA. ICA were detected in 32 (57.1%) of the 56 patients.

It seems that HTLV-I is playing no role in IDDM.

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EPIDEMIOLOGIC AND MOLECULAR CHARACTERIZATION OF NEW HTLV-I INFECTION FOCUS IN THE MIDDLE EAST

Yehuda L. Danon, Yael Kilim and Joseph Rosenblatt, Kipper Institute of Child Immunology, Children's Medical Center of Israel, Sackler School of Medicine, Tel-Aviv University Israel

HTLV-I has been linked to adult T-cell leukemia/lymphoma (ATLL), and HTLV-II to rare cases of chronic T-cell leukemia. We recently reported a high rate of HTLV-I sero-positivity among immigrants to Israel from Mashad in Northeastern Iran after a national serologic survey of blood donors. We have now characterized the Iranian HTLV-I isolate using a combination of Southern blotting, polymerase chain reaction (PCR) and sequencing. DNA from 17 HTLV-I seropositive Mashadi carriers was isolated from peripheral blood mononuclear cells and amplified by PCR using primers to the tax-rex region. All 17 samples were found to be HTLV-I and not HTLV-II using discriminatory PCR. The cell line contained integrated HTLV-I provirus. Southern blotting and restriction mapping of the Iranian isolate demonstrated marked similarity to published Japanese isolates using 10 different restriction enzymes. Amplification and preliminary sequencing of a 900 bp segment of env from two Mashadi isolates disclosed approximately 95% nucleic acid sequence homology with the published sequence of Japanese isolates, and >98% homology between two Mashadi isolates. These data indicate substantial conservation of sequence between Iranian, African (Zair) and Japanese HTLV-I isolates. Insights derived from the study of HTLV- I/II can be applied to the search for other retroviruses and oncogenic viruses.
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A Decade with HTLV-I/HTLV-II: Lessons in Viral Leukemogenesis

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INTRODUCTION

The past decade has seen myriad advances in detection and characterization of human retroviruses. It began with initial description of human T-cell leukemia virus type I (HTLV-I) by Poiesz and Gallo in the US and Yoshida in Japan, which pointed to the involvement of the human retrovirus, HTLV-I, in an unusual form of T-cell malignancy, adult T-cell leukemia/lymphoma (ATLL) (1,2). The identification of HTLV-I intensified the search for related viruses, and soon thereafter, human T-cell leukemia virus type II (HTLV-II) was described by Kalynaraman and Gallo in a cell line derived from a patient with a chronic T-cell leukemia with features of hairy-cell leukemia (3). The rapid identification of HTLV-II on the heels of HTLV-I led to speculation that a host of human oncogenic retroviruses would soon be identified. The subsequent discovery of human immunodefiency virus types 1 (HIV-1) and 2 (HIV-2) and their implication in acquired immunodeficiency syndrome (AIDS) accelerated the pace and intensity of the search for oncogenic viruses. It was soon recognized that leukemic cells in malignancies associated with HTLV-I and -II contained clonally integrated provirus; in effect, a signature for direct viral involvement in the oncogenic process. In contrast, neoplasms frequently seen in the setting of HIV-1 infection (e.g. Kaposi's sarcoma and/or high-grade B-cell lymphomas) did not appear arise as a direct consequence of viral transformation of HIV-1-infected cells. At the end of the decade, only HTLV-I and -II remain clearly implicated as directly leukemogenic human retroviruses. Therefore, we believe that insights gleaned from investigation of these viruses can and should be applied to the search for other oncogenic retroviruses.

EPIDEMIOLOGICAL LESSONS

The epidemiological link between HTLV-I and ATLL resulted from two complementary events. The first, an appreciation by Uchiyama and Takatsuki that ATLL represented a unique clinical entity (4) allowed geographic localization of the disease to southern islands of Japan: Kyushu, Shikoku, and the Ryuku chain of islands. Development of serological assays for HTLV-I led to correlation of HTLV-I infection to the presence of malignancy, as well as a determination of modes of transmission (for review see 5). Epidemiological studies have suggested that exposure shortly after birth is a major risk factor for subsequent development of ATLL (5,6). In addition, these studies have demonstrated that twenty or more latent years may elapse between acquisition of infection and development of malignancy (5,6). Furthermore, only a minority (< 5%) of HTLV-I carriers actually develop ATLL (7), and ATLL as a consequence of transfusion-acquired HTLV-I is virtually unknown.

Hence, several general observations emerged from scrutiny of HTLV-I epidemiology: (a) leukemia may be an infrequent consequence of exposure to a fairly wide-spread virus; (b) leukemogenesis may depend on the timing and/or length of exposure, so that individuals infected in childhood may be at higher risk than those infected later in life; and (c) the long latency period suggests a multiple step process may be involved in leukemogenesis; while viral infection may be a prerequisite, it alone may be insufficient to produce the leukemic phenotype. These general epidemiologic features of ATLL suggest that a systematic re-evaluation of appropriate candidate malignancies and viruses may reveal other oncogenic viruses. Fairly prevalent or even ubiquitous viruses could conceivably manifest oncogenic potential in a sporadic fashion, and factors such as timing and length of exposure may be critical.

Careful cataloguing and description of clinical syndromes is essential to derive epidemiologic clues that may lead to virus identification. The recognition that non-Hodgkin's lymphomas could be divided into T- and B-cell subtypes and subsequent differentiation of ATLL from *mycosis fungoides* is a case in point. While ATLL was undoubtedly a frequent reason for in-patient hospitalizations in Japan prior to 1977, it was thought to be a variant of peripheral cutaneous T-cell lymphoma, and its characteristic features such as hypercalcemia and enhanced expression of interleukin 2 (IL-2) receptor alpha (IL-2Rα) chain (Tac antigen) on the cell surface were initially overlooked. Recognition of the subtler clinical aspects of the syndrome allowed for subsequent epidemiologic observations (4).

In contrast to HTLV-I, it is premature to reach conclusions regarding pathogenesis by HTLV-II. Although originally isolated from the Mo T-cell line, a transformed T-cell line derived from the spleen of a patient with hairy-cell leukemia, the nature of the malignancy *in vivo* in the patient was not adequately addressed (8). We know that HTLV-I and -II can transform T-cell lines *in vitro*, and that the Mo T-cell line may have simply represented an outgrowth of HTLV-transformed cells *in vitro*. A second patient with HTLV-II and hairy-cell leukemia was found by our laboratory to have a biclonal lymphoproliferative

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LEUKEMIA

disorder in which a B-cell hairy-cell leukemia and a co-existant malignant CD8+ T-cell clone were observed (9,10). Oligoclonal integration of HTLV-II provirus into the CD8+ T-cells provided strong evidence for origin of malignancy in a virally infected cell. However, as additional cases of HTLV-II-induced malignancy have not been reported, there is considerable doubt as to whether we have as yet characterized the prototypic disease associated with HTLV-II.

An additional surprise that has emerged from epidemiological studies of HTLV has been the fact that screening procedures for HTLV-I identify a considerable number of crossreactive HTLV-II carriers. This raises the possibility that in the process of assaying for newly identified viruses, we may inadvertantly be assaying for a variety of crossreactive members of the same viral family. Specifically, intravenous drug abusers (IVDA) found to be seropositive for HTLV-I have been reported in several studies to have a higher incidence of HTLV-II infection and > 50% of seropositive random blood donors screened by HTLV-I ELISA were actually found by DNA amplification techniques to harbour HTLV-II (11–13). In the future, screening for newly identified viruses should be performed using both DNA amplification and serological techniques to avoid the initial confusion in delineating the epidemiology of HTLV-I and -II.

The HTLV-I model of malignancy as a rare consequence of infection with a prevalent virus suggests that careful molecular re-examination of the role of already recognized viruses in suspect malignancies is in order. In the case of HTLV-I, over a million infected individuals in Japan give rise to only 400–500 cases of ATLL per year. Hodgkin's disease (HD) may provide another case in point. The bimodal age distribution, prevalence, anecdotal descriptions of geographical clustering, and 'outbreaks' of HD suggest that an infectious agent may underlie pathogenesis (14–21). The increasingly frequent reports of Epstein-Barr virus (EBV) genome detection in some cases of HD suggest that EBV can be important in pathogenesis of a subset of HD patients (14–21). As another example, recognition that four of the last five cases of ATLL-like T-cell lymphoma in Israel occurred in Iranian immigrants from the northeastern city of Mashad, allowed identification of a new focus of HTLV-I infection (22,23). Recognition of geographic, familial and/or ethnic clustering of particular malignant disorders may yield important clues to viral etiology. It is important to note, however, that such time/space clustering may frequently relate to non-infectious risk factors rather than a virus. Some investigators have speculated on the likelihood of viral involvement in childhood acute lymphoblastic leukemia (ALL); however, the evidence is only mildly suggestive at best. Some studies suggest an association with geographic areas of high socioeconomic status, while others do not (24–29). Additional studies suggest a mild degree of clustering, particularly in children less than six years of age, although this remains controversial (29–31). Thus, little evidence points to an infectious cause or an underlying common leukemia virus in ALL. If a virus were involved, analogy to ATLL would suggest that it might initially cause an insignificant acute infection that establishes latency and eventually leads to leukemia through secondary events. Given the lack of overt clustering, seroepidemiological studies are unlikely to settle the issue, and frank demonstration of molecular involvement of an infectious agent will likely be necessary.

New Molecular Mechanisms of Pathogenesis

The explosive growth in the study of oncogenes over the past decade came about as a result of recognition that in animal malignancies brought about by retroviral infection, the transduction of a cellular proto-oncogene and its inappropriate expression under control of the viral promoter was frequently observed in retrovirally induced tumors. A second type of molecular lesion frequently observed was integration of a retrovirus adjacent to a cellular oncogene and loss of normal patterns of proto-oncogene expression. These observations led to speculation that similar mechanisms may be operative in human malignancy. However, HTLV-I afforded a unique surprise, in that the viral sequences did not contain any transduced cellular sequences, and that integration sites appeared to be random. While clonal integration was observed by Yoshida and colleagues, the sites of integration often occurred on different chromosomes, and no specific integration patterns could be observed (2,32). These observations led to a search for new mechanisms of oncogenesis.

The demonstration by Seiki and colleagues of the unique coding regions located in the 3' end of the genome provided a first clue as to potential mechanisms (33). The 3' ends of the genome of HTLV-I, -II and the leukemogenic bovine leukemia virus (BLV) all contain coding sequences for at least two overlapping genes (34–37). These genes, known as *tax* and *rex*, are now known to encode a transcriptional and post-transcriptional regulator of viral expression. The initial discovery of the *tax* gene was surprising, in that such *trans*-acting transcriptional regulators had only been previously identified in DNA viruses such as herpes simplex virus, varicella-zoster virus, and adenovirus. The HTLV-I *tax* gene encodes a 40-kilodalton (kDa) protein, and the HTLV-II *tax* gene encodes a 37-kDa protein (34–37). These nuclear phosphoproteins are highly homologous, and have similar effects on transcription. Tax expression is necessary for transcriptional activation of virus replication and efficient RNA production from the viral long terminal repeat (LTR); however, an additional property noted early was the ability of Tax to *trans*-activate other viral and cellular promoters, including those involved in regulation of T-cell growth. Examples of such promoters include those for IL-2, IL-2Rα, and the lymphokine, granulocyte-macrophage colony-stimulating factor (GM-CSF) (38–41). In the case of HTLV-I and -II, Tax appears to interact with three 21-base pair (bp) repeats located in the U3 portion of the viral LTR. Within the sequence of the 21-bp repeats are core sequences known to bind cellular transcription factors (42,43). A variety of these proteins have now been identified and

partially characterized.

In contrast to the HTLV promoters, Tax activation of the IL-2Rα gene involves induced nuclear expression of a cellular DNA binding protein, which interacts with an NF-κB-like enhancer (44). NF-κB is a DNA-binding factor first shown to interact with the enhancer of the κ light chain immunoglobulin gene (45). Tax interaction with NF-κB is thought to account for inducibility of some other cellular and viral promoters such as the HIV-1 promoter. The lack of an NF-κB-like binding site in the HTLV-I/-II promoter and deletion of NF-κB-like binding sites from the GM-CSF promoter with retention of response to Tax indicate that Tax may act via different pathways in different cellular and promoter contexts (41).

To date, only limited evidence directly implicates Tax in T-cell transformation. Introduction of Tax coding sequences under the control of a herpes saimiri vector has resulted in continuously proliferating T-cell lines *in vitro*, although the transformed cell lines appear to retain dependence on IL-2 for continued growth (46). Expression of HTLV-I Tax under the control of the HTLV-I LTR in transgenic mice does not lead to Tax expression in T-cells, and T-cell malignancy is not observed (47). Some mice developed mesenchymal tumors reminiscent of neurofibromatosis, as well as muscular atrophy. Recent HTLV-I Tax transgenics under control of the T-cell-specific Thy-1 promoter also did not result in T-cell malignancy (48). Nevertheless, the promiscuous interaction of Tax with a variety of viral and cellular promoters suggests that it may play a pivotal role not only in the HTLV-I life-cycle, but also in definition of the malignant phenotype. Tax can *trans*-activate the IL-2Rα gene, which offered an explanation for the high degree of Tac (high affinity IL-2 receptor) antigen expression in HTLV-I-transformed T-cells. Similarly, ectopic GM-CSF production due to Tax may cause eosinophilia, which is frequently seen in ATLL. In addition, Tax may also be involved in *trans*-activation of the parathyroid hormone-related protein (PTHRP) promoter, perhaps accounting for the ectopic expression of PTHRP in ATLL cells, thereby leading to altered calcium metabolism (49). However, HTLV-I mRNA expression in ATLL is so low that it has required use of RNA polymerase chain reaction (PCR) to be detected. Therefore, whether effects seen with Tax *in vitro* have applicability to HTLV-I *in vivo* remains unclear.

The ability of the viral *trans*-activator, Tax, to interface with several cellular transcriptional factor pathways suggests a new model for viral leukemogenesis. The presence of such *trans*-acting genes may allow development of new assays for the presence of as yet undiscovered retroviruses based on the ability of *trans*-acting 'Tax-like' transcriptional regulatory proteins to act on cellular and viral genes. Models for cooperation between oncogenes could undoubtedly be applied to help dissect a potential role for Tax in cooperation with other oncogenes. New models for oncogenic cooperation have emerged at this conference, such as superinfection of E_μ-*myc* transgenic mice with Moloney murine leukemia virus (MoMuLV) (50,51).

Several conclusions can be derived from study of the *trans*-regulatory *tax* gene: (a) new mechanisms of retroviral leukemogenesis other than transduction of cellular proto-oncogenes and/or retroviral insertion adjacent to cellular proto-oncogenes may be operative in human malignancy; (b) human retroviruses possess transcriptional activators that may affect expression of cellular genes, and aberrant expression of cellular genes may contribute either to leukemogenesis *per se*, or to the leukemic phenotype; and (c) the effect of viral transregulatory genes may be felt early in leukemogenesis, and may be insufficient to elicit the full-blown leukemic phenotype.

An additional transregulatory gene studied more recently is the *rex* gene of HTLV-I, -II, and BLV. The *rex* gene is required for productive HTLV-I/-II infection. The *rex* gene of HTLV-I encodes two proteins, one of 27 kDa and one of 21 kDa, from an overlapping reading frame to that encoding $p40^{tax}$ (52). These proteins appear to result from utilization of an alternative initiator methionine. In HTLV-II, two proteins are also encoded of the apparent sizes, 26 and 24-kDa (53). In HTLV-II, these appear to derive from different degrees of phosphorylation, with the larger molecular weight species being a hyperphosphorylated form of the 24-kDa protein (54). In both HTLV-I and -II, the proteins appear to act as post-transcriptional regulators, and elicit export of full-length *gag/pol* mRNA and probably partially spliced *env* transcripts from cell nucleus to cytoplasm. The *rex* gene appears necessary to allow expression of non-spliced and partially spliced viral mRNA, which in turn allows synthesis of Env and Gag proteins and production of mature virions.

In HTLV-I, Rex has been found to act through a *cis*-acting Rex-responsive element (RxRE) located in the 3' LTR. Our group has studied Rex effects mediated through the 5' LTR of HTLV-II (55). In both cases, Rex appears to act through sequences located in the R region, downstream from the transcription initiation site. Assays of binding to radiolabeled viral RNAs have demonstrated that purified HTLV-II Rex can directly bind to transcripts initiated from the 5' LTR, and that binding occurs to a portion of a *cis*-acting element responsible for Rex action, known as the RxRE (56,57). Mapping in our laboratory has demonstrated that Rex can bind directly to transcripts as short as 115 bp derived solely from sequences within the R region (57,58). These transcripts contain the 5' LTR splice donor site, and mutation of the splice donor site appears to impair Rex binding and function. Furthermore, Rex binding is dependent on retention of a specific stem-loop mRNA structure located downstream from the splice donor site (from nucleotide 465–501 within the HTLV-II 5' LTR) (57). This stem-loop structure is conserved in both HTLV-I and -II. Rex binding may be facilitated by hyperphosphorylation, and it would appear to be the higher molecular weight (26 kDa) Rex species of HTLV-II that binds efficiently, indicating that cellular controls on Rex function may exist at the level of phosphorylation

(Chen and Green, unpublished observations). Nucleolar localization and our results using RxRE mutations of the splice donor site suggest a direct interaction of Rex with the cellular splicing apparatus to facilitate bypass of cellular splicing mechanisms.

An intriguing observation regarding Rex of HTLV-I was first made by Rimsky and Greene, demonstrating that HTLV-I Rex can functionally substitute for the Rev protein of HIV-1 (59). The Rev protein of HIV-1 performs an analogous function to that described for Rex in HTLV-I. Their assay demonstrated the capacity of Rex to induce production of the truncated single-exon form of the HIV-1 Tat protein that reflects translation from unspliced *env* vector mRNA (57). HTLV-II Rex in our laboratory is also able to rescue replication of Rev-deficient mutants of HIV-1 (58). Rescue of HIV-1 Rev-deficient mutants by HTLV-II Rex is relatively inefficient, and this can partially be accounted for by the relatively low affinity of HTLV-II Rex binding to the HIV-1 Rev-responsive element (RRE) (58). In addition, HIV-1 Rev is unable to complement an HTLV-II Rex-deficient clone, indicating a non-reciprocal pattern of complementation. Nevertheless, the ability of Rex to complement the genetically distant HIV-1 virus in *trans* suggests that, like Tax, Rex may act promiscuously on a variety of non-HTLV target sequences. This raises the possibility that Rex may also interact with cellular RNA to elicit aberrant splicing and/or transport. Disrupted processing and expression of cellular mRNAs could conceivably be implicated in the process of leukemogenesis as well. Therefore, post-transcriptional regulators may also be involved in the process of retroviral leukemogenesis. Direct evidence supporting this hypothesis has not been obtained.

Disparate Disease Entities Related to HTLV-I

Approximately five years following its discovery, it was found that the pathology elicited by HTLV-I in one setting may not predict other forms of pathology related to the virus. A specific illustration is afforded by the two major non-overlapping syndromes associated with HTLV-I. While HTLV-I may cause ATLL, another subset of infected individuals, approximately half as many, will develop a slow neurologic disease characterized by gradual development of spastic paraparesis of the lower extremities with minimal sensory loss. This illness, tropical spastic paraparesis (TSP) (also known as HTLV-I-associated myelopathy (HAM)), is distinguished from multiple sclerosis (MS) by virtue of its chronic progressive and non-episodic nature, as well as the general limitation of pathology to motor control in the lower extremities and sphincter dysfunction. The association between this illness and the virus was discovered by Gessain and co-workers while screening neurologic illnesses for retroviral involvement in Martinique (60). As opposed to the leukemia, where HTLV-I has been observed to infect and transform T-cells *in vitro*, no adequate model for pathogenesis of the myelopathy exists. Regardless of underlying mechanisms, involvement of HTLV-I in a slow neurologic disease was not predictable on the basis of its involvement in T-cell leukemia. The latency period for development of HAM is also appreciably shorter, and recently at UCLA, we saw a patient develop myelopathy approximately fifteen months following infection by transfusion (61). In contrast, development of ATLL following transfusion-acquired HTLV-I is almost never seen. Furthermore, co-existent ATLL and HAM have rarely been described. We have observed at least one case of multiple members of an Iranian Jewish family developing HAM (D. Meytes *et al.*, unpublished), and this has been reported by other investigators. This would suggest that either differences in host genetic make-up and susceptibility or differences in viral isolates may account for familial HAM. It is important to note that the association between HAM and HTLV-I was made serendipitously. Quite possibly, if the link to HAM had been described first, no search for HTLV-I association with malignancy would have been made. This would suggest that other viruses that may not be associated in investigators' minds with development of malignancy may be candidates for potential oncogenic roles. Good candidates would be viruses with *trans*-acting transcriptional proteins, such as members of the herpes family, adenovirus, and/or other retroviruses.

DISCUSSION

Over a decade following initial identification of the role of the retrovirus in human disease, HTLV-I remains as the only example for human retroviral leukemogenesis. Several lessons pertinent to the search for leukemogenic viruses can be derived from the HTLV model. First, leukemia may be the rare consequence of infection with a relatively common virus. Second, a long latency period may be involved following acquisition of infection, and the leukemogenic process may involve multiple steps beyond initial infection. Third, the mechanism by which the virus may predispose to development of leukemia may be different from that seen with other retroviruses, and may involve *trans*-acting viral genes, either regulators of DNA transcription or regulators of viral mRNA expression. Finally, the role of HTLV-I as an etiologic agent in both HAM and ATLL suggests that viruses not thought to have oncogenic properties may on occasion predispose to malignancy. The overall lesson from the HTLV experience is that when suitable or suggestive epidemiologic factors are found, such as ethnic, geographic and/or familial clustering in the absence of a known inheritance pattern, a search for underlying viruses should be initiated. The search would involve removal of malignant tissue, when possible, for culture *in vitro* and a search using available molecular and serologic probes. The success in identifying HTLV-II by virtue of limited nucleic acid homology and antigenic similarity to HTLV-I suggests that new retroviruses can be identified. The demonstrated crossreactivity between HTLV-I and -II suggests that any search should be accompanied by rapid isolation of nucleic acid probes for viral sequences of interest, so that crossreactive

entitites can be discerned. A fresh look should be taken using newly available probes as a means of determining viral clonality, particularly for DNA viruses such as herpesviruses to assess whether a particular malignant tissue has arisen from a single virally infected cell. Furthermore, scrutiny of viruses already known to be widespread in the population may prove fruitful, as already appears to be the case for EBV and a subset of Hodgkin's disease. A re-duplication of such efforts will determine whether new retroviruses with oncogenic potential will be identified in man in the upcoming decade, or whether HTLV will remain an isolated if fascinating example of retroviral leukemogenesis in man.

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Immunoglobulin Prophylaxis against HTLV-I in a Rabbit Model

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We have investigated the protective effect of human T-cell leukemia virus I (HTLV-I) immune globulin (HTLVIG) against HTLV-I in rabbits. HTLVIG containing 77 mg/ml of IgG was prepared from pooled plasma from seropositive healthy persons. In the first experiment, four groups (A, B, C, and D) of three rabbits were transfused with 5 ml blood from an HTLV-I-infected rabbit. Groups A, B, and C were infused 24 h later with 10, 5, and 2 ml HTLVIG, respectively, while group D was infused with 10 ml HTLVIG 48 h later. Seroconversion for HTLV-I occurred in none of group A, one of group B, and all of groups C and D after 2–5 weeks. In the second experiment, four litters (E, F, G, and H) born to another virus-infected rabbit and consisting of 7, 5, 7, and 7 newborns, respectively, were used. Litters E and H were allowed to grow normally as controls, while litters F and G were given intraperitoneal inoculation of 3 ml/kg of HTLVIG weekly four times until weaning. Although three of litters E and H each seroconverted after 5–8 weeks, none of litters F, and one of litter G became antibody-positive after 10 weeks. Presence or absence of HTLV-I infection in all these animals was confirmed by transfusion assay or gene amplification. These results indicate that passive immunization protects rabbits against blood- and milk-borne transmission of HTLV-I.

INTRODUCTION

A rabbit model of human T-cell leukemia virus I (HTLV-I) infection has been established, in which the virus was shown to be transmissible not only by blood transfusion (1,2) but also from dam to offspring via milk (3,4). In the blood transfusion experiment, as little as 0.01 ml blood from a virus-infected rabbit was capable of transmitting HTLV-I (2). Furthermore, milk or semen lymphocytes from seropositive healthy persons transmitted HTLV-I when inoculated intravenously into rabbits (5). This animal model, therefore, provided a unique opportunity to study the protective effect of passive immunization against HTLV-I (2,6). In the present experiment, immunoglobulin prophylaxis against blood- and milk-borne transmission of HTLV-I was further explored.

MATERIALS AND METHODS

Rabbits

Japanese white rabbits, weighing about 3 kg, purchased from a commercial breeder were used.

Detection of Antibodies to HTLV-I

Blood samples were taken from rabbits at intervals of 1–2 weeks and sera were titrated for HTLV-I antibodies by indirect immunofluorescence against the MT-2 cell line as described previously (2). The presence or absence of immunoglobulin G (IgG) antibodies was verified by Western blot using a MT-2 lysate as antigen. Sera were also tested for IgG and immunoglobulin M (IgM) antibodies by enzyme-linked immunosorbent assay (ELISA) against disrupted HTLV-I virions according to the manufacturer's instructions (Eisai, Tokyo). Neutralizing antibodies were assayed against vesicular stomatitis virus (VSV) bearing envelope antigens of HTLV-I as previously described (7).

HTLV-I Immune Globulin (HTLVIG)

HTLVIG containing 77 mg/ml of IgG was prepared from pooled plasma from seropositive healthy persons by the method of polyethylene glycol fractionation (8). The preparation had an immunofluorescence anti-HTLV-I titer of 1:5120 and a VSV (HTLV-I) pseudotype neutralizing antibody titer of 1:6250.

Transfusion Assay

To ascertain the status of HTLV-I infection, 20 ml of blood obtained from experimental rabbits were transfused into normal rabbits. Seroconversion of the recipient rabbits indicated a virus carrier state of the donor rabbits.

Polymerase Chain Reaction (PCR)

DNA extracted from peripheral blood mononuclear cells was analyzed for the presence of HTLV-I sequences by the method of Kwok *et al.* (9). DNA, 1 μg, was subjected to 40 cycles of denaturation followed by annealing and extension. Oligonucleotide primers at 7341–7360 and 7460–7411 corresponding to the pX region of HTLV-I were used. Amplification was performed using a thermostable DNA polymerase on an automated DNA Thermal Cycler (Perkin-Elmer/Cetus, Norwalk, CT). The amplified products were electrophoresed on 6% polyacrylamide gels, transferred to nylon membranes, and hybridized with a ^{32}P end-labeled probe at 7364–7383.

RESULTS

Passive Immunization against Blood-borne Transmission of HTLV-I

Four groups (A, B, C, and D) of three rabbits were first transfused with 5 ml of blood from an HTLV-I-infected rabbit. Groups A, B, and C were infused 24 h later with 10, 5, and 2 ml HTLVIG, respectively, while group D was infused with 10 ml HTLVIG 48 h later.

Seroconversion for HTLV-I occurred in none of group A, one of group B, and all of groups C and D after 2–5 weeks (Figure 1). All five rabbits which were protected from seroconversion remained seronegative during an observation of six months. Sera taken immediately after infusion of HTLVIG showed anti-HTLV-I titers of 1:320 for groups A and D, 1:80 for group B, and 1:20 for group C. The VSV (HTLV-I) pseudotype neutralizing titers of these sera were 1:1250

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LEUKEMIA

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Serological and molecular survey for HTLV-I infection in a high-risk Middle Eastern group

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To define the extent of human T-cell leukaemia virus (HTLV-I) infection among a group of Jewish immigrants to Israel with an increased frequency of adult T-cell leukaemia, various serological and molecular screening methods, including enzyme-linked immunosorbent assay (ELISA) for anti-HTLV-I, ELISA for antibody to recombinant HTLV-I p40*tax* protein, and molecular detection of infection by polymerase chain reaction (PCR) amplification of HTLV-I proviral DNA from peripheral blood mononuclear cell DNA, were used. By HTLV-I ELISA the overall rate of infection was 12% (24 of 208) among immigrants from Khurusan, northeastern Iran; no HTLV-I carriers were detected among 111 unselected Jewish immigrants from other parts of Iran. There was unexplained clustering of HTLV-I infection within a cohort of 32 elderly women of similar geographic origin in a home for old people—14 were seropositive by ELISA and 19 of 29 were positive by PCR. The findings in this newly identified high-risk population suggest that in addition to ELISA, other screening techniques may be required to detect all carriers in high-risk populations.

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Introduction

Human T-cell leukaemia virus type I (HTLV-I) infection has been described in southern Japan, the Caribbean basin, and the northern parts of South America, and in certain high-risk groups, such as intravenous drug abusers in the United States.[1,2] Previous reports of HTLV-I infection among Ethiopian Jews in Israel were not confirmed.[3,4] During the past 4 years, sporadic cases of adult T-cell leukaemia linked to HTLV-I have been reported in Israel[5–11] and 1 of the 5 latest cases were among immigrants to Israel who originated from the city of Mashad in northeastern Iran.[11] Because of these findings, we undertook a systematic survey of Iranian Jews in Israel, focusing on immigrants with links to Mashad.

Subjects and methods

Blood samples from Israeli blood donors of Iranian origin were obtained from the Israeli Magen David Adom Blood Services Center, Tel Aviv. The criterion for classification as an Iranian control was that the country of birth of the blood donor or at least one of his or her parents was Iran. Blood samples were collected on three occasions from residents of a Mashadi home for elderly women in the Tel Aviv area and from three Mashadi community synagogues in the cities of Bnei Brak and Tel Aviv. Samples were classified as Mashadi if the donor or at least one of his or her parents originated from Mashad, Iran. 20 samples from patients on long-term haemodialysis, 8 from patients with T-cell malignant disorders other than adult T-cell leukaemia, and 12 from Ethiopian Jewish immigrants were also included.

Serological screening was done for HTLV-I antibodies on serum or plasma samples by means of an enzyme-linked immunosorbent assay (ELISA; Abbott Laboratories). Confirmatory western blotting and or radioimmunoprecipitation assay (RIPA) with sulphur-35-labelled methionine HTLV-I-infected HUT 102B

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PREVALENCE OF HTLV-I INFECTION

	HTLV-I ELISA and western blot		Anti-p40*tax* ELISA	
	No tested	No (%) positive	No tested	No (%) positive
Mashadi Jews	208	24 (12%)	127	12 (9%)
Other Iranian Jews	111	0	20	0
Ethiopian Jews	12	0	12	0
Haemodialysis patients	20	0	20	0
Patients with T-cell malignant disorders	8	0	ND	

lysate were also done.[11] Samples positive in the ELISA were tested by both confirmatory methods. Antibodies to HTLV-I p40*tax* were measured by means of an ELISA with recombinant p40*tax* as antigen on the solid phase (polystyrene beads) (Abbott). HTLV-I seropositive infected samples with known reactivity against p40*tax* on RIPA were used as positive controls, and 4 samples negative for HTLV-I on ELISA and western blot as negative controls. Samples were scored as positive for p40*tax* if the optical density exceeded 4·5 times the mean negative control value. The polymerase chain reaction (PCR) was used to amplify HTLV-I sequences of DNA from peripheral blood mononuclear cells with primers to a 159 bp segment contained within the *tax/rex* gene as previously described.[12]

Results

The extensive social and ethnic ties between Jews of Mashadi origin allowed selective sampling of this population within Israel. On repeated assays, 24 of 208 (11·5%) Mashadi serum samples were positive by ELISA for antibodies to HTLV-I and were confirmed by western blotting. In contrast, none of the 151 control samples was positive by ELISA (table).

In an effort to survey the oldest members of the Mashadi population, we tested 32 unrelated elderly Mashadi women living in an old people's home in Tel Aviv. 14 (44%) were seropositive, a rate three times higher than that in the general Mashadi population. At our first sample collection in 1988, 12 (52%) of 23 long-standing residents of the home were seropositive; 2 of 9 new residents surveyed a year later were seropositive. Of 26 Mashadi women older than 60 years who lived elsewhere in Israel only 3 (12%) were seropositive.

To determine whether serological assays had detected all infected subjects, we carried out PCR amplification of HTLV-I DNA from 29 of the residents of the old people's home. 19 were positive for HTLV-I sequences: 14 of these were seropositive, 4 were seronegative, and 1 was seronegative by standard ELISA but reactive in the anti-p40*tax* ELISA. A second PCR on repeat samples from 10 of these women showed concordant results in 6 of 6 PCR-positive and 2 of 3 PCR-negative cases; 1 woman originally positive on PCR was negative on the second sample, and 1 woman originally PCR-negative was faintly positive on her second sample and had antibodies to p40*tax* by ELISA. This unusual clustering of HTLV-I infection within the old people's home suggested acquisition of infection within the institution.

In addition to antibodies to virion components, we assayed for antibodies to the non-structural p40*tax* protein. Of 128 Mashadi samples tested 12 had absorbance levels 4·5 or more times those of the negative control and were judged positive (table). 103 samples were negative by both assays. 8 samples were seropositive for both anti-HTLV-I and anti-p40*tax*; 13 samples were positive for anti-HTLV-I and negative for anti-p40*tax*; and 4 were positive for anti-p40*tax* but repeatedly negative for anti-HTLV-I by ELISA and western blot. 3 of the latter group of samples were negative on PCR, and the other, from a resident of the old people's home, was positive. All 4 were negative by western blotting and by RIPA. Therefore, the reason for the high titres in three samples in the anti-p40*tax* ELISA is unclear.

Direct comparison of anti-p40*tax* ELISA results and those of the RIPA was done for 28 subjects. 8 samples were positive for anti-p40*tax* by both RIPA and ELISA; 1 with traces of antibody by RIPA was negative by ELISA; the 3 samples mentioned previously were negative by RIPA but repeatedly positive by ELISA; and 16 were negative by both tests. The usefulness of the anti-p40*tax* ELISA in detecting true additional HTLV-I-infected seronegative individuals remains unclear, since seronegative infection was corroborated by other means of detection in only 1 of the 4 questionable cases.

The finding of a higher rate of seropositivity among elderly Mashadi women than in the general Mashadi population suggested a potential for gradual development of seropositivity with age, as has been reported by others. Because of the possibility that serological screening may not detect all positive subjects' we carried out HTLV-I-specific PCR on DNA from 68 randomly selected Mashadi blood samples. 5 of 7 known seropositive samples were positive in the PCR assay, and 61 seronegative samples were all negative by PCR. Hence, PCR did not increase sensitivity of detection within this sample above that achieved with serological assays alone. All PCR-positive samples contained HTLV-I and not HTLV-II, as shown by discriminatory PCR (data not shown).

Discussion

We have identified a high risk of HTLV-I infection in Iranian Jews originating from the city of Mashad in Khurusan, northeastern Iran. This group seems to have an overall infection rate of about 12%, though the rate was much higher among residents of a Mashadi home for old people. The reason for this clustering is unclear, although the findings suggest acquisition of infection within the institution. The overall level of infection among other Iranian Jews seems to be substantially lower than that among Mashadis. The explanation may be geographic, and Mashad may be within a previously unrecognised endemic region. Since we did not test non-Jewish Mashadis, this possibility cannot be excluded.

The unique history of the Mashadi community may help to explain their high rate of HTLV-I infection. In 1839, the Jewish community in Mashad was subjected to a series of violent attacks and forced to convert to Islam, though the majority of the community continued to practise Judaism covertly.[13] To safeguard the community secret necessitated a very high rate of intermarriage among community members. Members of this community continued to marry close relatives over the next 150 years. Markers of consanguinity are high among Mashadi Jews—for example, over 20% have a deficiency of glucose-6-phosphate dehydrogenase (D. M., unpublished). The chance introduction of HTLV-I infection into the community could have resulted in rapid transmission of the virus by sexual means and by breastfeeding.

An estimated 5000–6000 Mashadis now live in Israel. This is the first Israeli ethnic group identified as having a high rate of HTLV-I infection. Members of the Mashadi Jewish community also migrated to the USA and parts of

Europe. We predict that the rate of infection among these migrants would be similar to that of the Israeli cohort.

In this study, we used several methods to detect HTLV-I infection. Our rate of anti-p40*tax* seropositivity in carriers was somewhat lower than that found by others among healthy HTLV-I carriers.[7,14,16] Serum samples from 4 subjects were positive only for anti-p40*tax* antibodies. Independent evidence of infection was obtained by PCR in only 1 of the 4. Thus, detection of anti-p40*tax* antibodies did not appreciably add to the estimate of the rate of infection. Our findings on the use of PCR suggested that in a high-risk population, such as the old people's home we studied or in families of HTLV-I carriers, PCR would increase the number of infected individuals above that detected by serological means. The usefulness of PCR as a screening assay in appropriate settings requires further study.

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Quinine-induced disseminated intravascular coagulation

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Recurrent disseminated intravascular coagulation occurred in 3 women after ingestion of quinine tablets for cramp. All had circulating quinine-dependent antibodies to platelets and in 2 there was initial evidence of antibody consumption, with low titres that rose steeply over the next few days and remained high for many months.

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Introduction

Recognised haematological problems associated with ingestion of quinine include thrombocytopenia, erythrocyte haemolysis, and neutropenia. Quinine was first implicated as a cause of purpura in the late 19th century,[1] and there have been several reports of associated thrombocytopenia.[2,3] However, we are aware of only two published cases of disseminated intravascular coagulation induced by quinine,[4,5] and report three further cases.

Patients and methods

Case histories

A 74-year-old woman was admitted 5 times over 3 years with various symptoms, which included acute shortness of breath, wheeze, generalised abdominal pain, fever, lower back and chest pain, melaena, haematemesis or haemoptysis, and bruising and petechiae. Most episodes occurred shortly after going to bed. Investigations on each occasion (table) showed evidence of disseminated intravascular coagulation (DIC). On the first 2 admissions she was treated with antibiotics, although blood cultures were always negative. On the third admission she was treated for asthma, and on the last 2 occasions no specific treatment was given. On each occasion, fever and other symptoms resolved within 24 h with a subsequent resolution of coagulation abnormalities. At least 2 similar, but milder, episodes also occurred for which she did not attend hospital. On 3 occasions recent quinine ingestion was clearly remembered by the patient or documented in the admission notes. A check on the number of tablets left in the bottle after her initial prescription indicated that 10 tablets had been taken over 7 years. Retrospective quinine-dependent platelet antibody analysis on samples stored from the last 3 admissions and from intervening periods showed low or undetectable antibody concentrations during the first 2 days of each acute episode, which then rose sharply

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MOLECULAR CHARACTERIZATION OF HTLV-I INFECTION IN ISRAEL

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Short Title: Characterization of Israeli HTLV-I isolate

INTRODUCTION

Many studies were performed on human T-cell leukemia virus type I, ethiologically associated with adult T-cell leukemia (ATL) [1,2] and HTLV-associated myelopathy (HAM).[3-7]

HTLV-I genomes isolated from ATL and HAM patients from different geographical origin demonstrated a high degree of homology (>96%).[5-8] Sequence variation in different isolates were found mostly in the LTR (1.3-5.2%) and the region between the envelope and tax/rex reading frames (0.1-6.9%).[6] It was found that genetic diversity between different isolates is in association with the geographical origin and not with the clinical presentation.[6,7,8] In 1988, HTLV-I was first discovered in Israel and the Middle East.[9] Two years later a community of jewish immigrants from the city of Mashad in northeastern Iran was identified with an infection rate of about 12%.[10]

Our aim was to determine the nucleotide sequence of LTR and env gene from HTLV-I genome of an HAM patient who originated from Mashad. This data was compared to sequences derived from HTLV-I isolated from Japanese and African patients in order to locate the origin of the Mashadi virus.

MATERIALS AND METHODS

Cell lines: Lymphocytes were collected from 20ml peripheral blood by a Ficoll-Hypaque density gradient and were resuspended in RPMI-1640

medium supplemented with 15% fetal bovine serum, interleukin-2 (IL-2) at a concentration of 50u/ml, 0.5% PHA and 1% penicillin/streptomycin. Cells were incubated at 37^{o}C in the presence of 5% CO2 and were maintained for several weeks. The cells were cryopreserved at various intervals for DNA extraction.

DNA extraction and PCR amplification: DNA was extracted from lymphocytes with phenol/chloroform. Two regions in the HTLV-I genome were amplified: LTR and env gene. LTR amplification was performed with the primers R11/14 which defined a 741 base sequence from nucleotide 61 to 802 (nucleotides are numbered according to the sequence of Seiki et al.[11] The env gene was amplified by two pairs of primers : R15/17A which defined a 1163 base sequence from nucleotide 5201 to 6364 and R19/18 primers which defined 791 base sequence from nucleotide 5942 to 6733.

The patient's (HE) DNA was PCR amplified for 30 cycles in a reaction containing 100 μl of 2mM MgCl, 200 ng from each primer, 0.2 mM from each dNTPs and 2.5 u of taq polymerase (USB, Cleveland, OH).
A list of primers used for PCR amplification is presented in table 1.

DNA sequencing: PCR products were recovered from PCR reaction with DS primer Remover (Advanced Genetic Technologies Corp, Gaithersburg, MD) and ethanol precipitation. Nucleotide sequence analysis was performed by the Taq Dye Deoxy Terminator Cycle Sequencing kit using the 373A DNA Sequencer (Applied Biosystems) at the Biological Services of the Weizmann Institute Rehovot Israel. Sequence analysis was performed at least twice for each primer (Table 1).

Sequence comparison: HE sequence was compared to the Japanese (ATK), African (EL) and Papua New Guinea (PNG-1) sequences by gcg program with the accession numbers: JO2029 (ATK), S74562 (EL LTR), M85207 (PNG-1) and M69044 (EL).

RESULTS

Comparison of HTLV-I (HE) LTR to other isolates. PCR amplification of HTLV-I LTR produced a PCR product of 741bp of which 698 bases from it were sequenced. 7 nucleotides which are not verified yet are designated as N. HTLV-I-(HE) LTR sequence was compared to three other isolates. The Japanese (ATK) sequence showed nucleotide homology of 97.8% with 15 nucleotide differences.[11] The African sequence showed nucleotide homology of 95% with 35 nucleotide differences and the Papua New Guinea isolate, which was compared only by 629 nucleotides, showed 91% homology with 56 nucleotides differences.[5,6]

Comparison of the sequence changes in the LTR region to the Japanese sequence showed that the differences are clustered mostly in the U3 (66.7%), 6.7% were in the R and 26.7% were in the U5 regions. There are no changes at the three 20bp enhancer elements at the U3 region and there is 98.7% homology for the Rex Responsive element located between bases 313 and 627.

Comparison of the env gene amplified from HTLV-I (HE) genome to the Japanese and African sequences. The PCR product was 1532bp in length from which 1496 bases were sequenced. The sequence between nucleotides 5625-5685 is not verified yet.

Comparison to the Japanese (ATK) sequence showed nucleotide homology of 97.7% with 9 nucleotide differences while the African (EL) sequence showed nucleotide homology of 98.4% with 6 nucleotide differences.[12,14] There is a problem with base T at position 5400. It seems that it creates a stop codon while in the other two sequences it does not exist. This finding needs further examination. HE env2 sequence was compared to the Japanese (ATK) sequence.[11] 98.7% homology at the nucleotide level with 12 nucleotide differences were found. Comparison to the African (EL) sequence showed nucleotide homology of 99.5% with 5 nucleotide differences.[12]

DISCUSSION

A new focus of HTLV-I infection was recently identified in the Middle East.[10] In this study we determined the sequence of two regions in HTLV-I isolated in Israel from an HAM patient belonging to this community: the LTR region and the env gene. Several studies have indicated a high degree of homology among HTLV-I isolates (>96%) and demonstrated that differences between variants are in association with their geographical origin.[5,6,7,8] Comparison of Mideastern sequence which originates in Iran to the Japanese, African and Papua New Guinea sequences in order to examine whether there is indeed a higher degree of homology between isolates from the same geographical area. A high level of homology, at about 98%, to the Japanese isolate, while the African and Papua New Guinea isolates were more divergent with sequence homology of 95% and 91%, respectively.

Comparison of HTLV-I env gene to the Japanese and African isolates showed homology at the nucleotide level of about 98-99%. From these results we can conclude that the env gene is more conserved than the LTR region.

With the exeptions of some nucleotides which are not verified yet, comparison of the LTR region showed a higher degree of homology between Iranian sequence and the Japanese isolate. As for the env gene, there is a high degree of homology between the Iranian sequence and the African isolate. With these results we cannot conclude if there is an influence of the geographical area on the virus genome.

There are two theories about HTLV-I origin and the way it was spread to the world. Gallo suggests that it originated in Africa and spread to the new world and Japan, while Saskena et al. suggest that HTLV-I originated in the Indo-Malay region.[5,13,14] Based on the comparison of the LTR sequences, our results support the theory of HTLV-I originating in Africa, since we found higher degree of homology between HE sequence and the African isolate, than with the sequence of Papua New Guinea isolate.

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Table 1: LIST OF PRIMERS FOR PCR AMPLIFICATION AND SEQUENCING

Primer	From nt.	to nt.	Used for	Seq.	Remarks
R11	61	77	amp. & Seq.	5' TAGAGCCTCCCAGTGAA	
R12	494	470	seq.	5' CCTAGACGGCGGACGCAG	Comp.
R14	802	786	amp. & seq	5' CTCGTATCCCGGACGAG	Comp.
R15	5201	5218	amp. & seq.	5' CATGGGTAAGTTTCTCGC	
R16	5660	5645	seq.	5' ATGGAGATTAATATTG	Comp.
R17	5641	5658	seq.	5' GCCTCAATATTAATCTCC	Comp.
R19	5942	5959	amp. & seq.	5' TCCATCCTCTTCTTCTAC	
R17A	6364	6347	amp.	5' TCCCAGAACAGGAGATCA	Comp.
R18	6733	6716	amp. & seq.	5' GGGAGAGGTAATTATTG	